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THE RÔLE OF THE LIPIDS IN ATHEROSCLEROSIS

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Atherosclerosis of the aorta and its branches, by far the commonest vascular disease, ranks high among the causes of natural death in man. Although the records and observations of atherosclerosis in man and animals have great antiquity, no generally acceptable cause for the lesions has been established. The tissue changes of atherosclerosis are regarded by some as part of an ageing process of the vessels, but according to the opinion of others the lesions are caused by lipid infiltrations of the intima and result from disorders of lipid metabolism.

Deposits of lipids, the formation of fibrous plaques and atheromas, and the deposition of calcium in the intima of the aorta and its branches are the dominant changes of atherosclerosis. The initial gross manifestations of the disorder are fatty streaks or small nodules in the root, arch and posterior wall of the aorta, especially about the ostiums of the intercostal arteries and other branches. Growths of fibrous tissue in these fatty deposits form larger discrete and confluent lesions. Later, with necrosis, the centers soften and become masses of soft lipid material and tissue debris, the atheromas. When the lining edge of the atheroma breaks, the lesion becomes an atheromatous ulcer. Simultaneously calcification may occur, and later even bone tissues may form. Injury of the lining of the blood vessels opposite these lesions favors thrombosis which increases the gravity of the atherosclerotic lesions. Complete descriptions of the gross and microscopic changes in aortic and arterial tissues with atherosclerosis have been published by Marchand (44), Duff (18), Leary (39, 40), Aschoff (4, 5) and Frey (21).

Many pathologists, among them Virchow (68), Sanders (58), Beitzke (9) and Wells (74) have stated that the development of atherosclerosis depends upon focal injuries or retrogressive changes of the media, commonly attributed to an aging process; the intima lesions, accordingly, being of secondary significance. Another view favored by Marchand (44), Lubarsch (9), Aschoff (4, 5), Anitschkow (2, 3), Leary (39, 40) and others has emphasized the importance of the lipid infiltrations into the intima. The lipid deposits, thus, are primary, initiate the other changes of the vessel walls, and atherosclerosis is a specific disease in which aging of the tissues is only a contributing factor.

Morphologic studies and chemical analyses of human and animal aortas have attempted to establish the significance of the lipids in atherosclerosis. The morphologic approach has demonstrated the presence of lipids, especially cholesterol, in the tissue lesions. Chemical analyses have revealed the composi-

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tion of these lipid deposits and have provided data for comparisons with the composition of lipids in other tissues of the body. The results of these chemical analyses also set apart the changes of atherosclerosis from those developing with senescence of tissues. Our review is limited to these chemical phases of atherosclerosis.

CHANGES IN THE CHEMICAL COMPOSITION OF THE AORTA WITH AGE. Atherosclerosis of the aorta and arteries is associated with an increased lipid content of the tissues. The chemical analyses demonstrating this increase are summarized by Wells (74). Because these analyses of entire aortas included both the normal and the atherosclerotic tissues, no distinction was made between the chemical changes occurring with the disease and those due to senescence. Weinhouse and Hirsch (71), in a study of these problems, quantitatively analyzed human aortas with and without atherosclerosis. To avoid the possibility of including pathologic tissues, only media, carefully dissected from the intima and adventitia, was used. The absence of retrogressive changes in the media was verified by microscopic examination.

TABLE 1

Variation in composition of media by age groups

AGE GROUP	MOISTURE*	LIPID EXTRACT†	CHOLESTEROL†			PHOSPHATIDE†			GALACTOSIDE†	CALCIUM†
			Free	Total	Ester	Ether soluble	Ether insoluble	Total†		
0–40	68.4	6.42	1.05	1.78	1.24	2.13	0.22	2.21	0.59	0.33
41–60	71.8	8.31	1.36	2.23	1.44	1.25	1.53	2.70	1.09	1.10
61–84	70.3	10.57	1.87	3.84	3.30	1.29	1.59	2.91	0.95	2.57

* The average content is given as percentage of wet tissue.

† The average content is given as percentage of dry tissue.

Lipids. A progressive increase in all lipid constituents of the media with age was observed (table 1). The free cholesterol in the highest age group was almost double the average value for the lowest age group. The cholesterol combined as ester in the highest age group almost tripled the amount in the lowest age group. The phospholipids as a whole increased only slightly with age. Within this class, however, there were relatively great changes. In the aortas of youths, ether-soluble phospholipids (lecithin and cephalin) preponderated; in the older aortas, ether-insoluble phospholipids (sphingomyelin) constituted the major portion of the phospholipid. The increase in the sphingomyelin fraction of the aorta from the lowest to the highest age group was great; from 0.22 to 1.6 per cent of the dry tissue. An increase with advancing age was observed also in the galactoside content; an observation in agreement with analyses by Kimmelstiel (32).

Calcium. The calcium content increased greatly with age from a low value of 0.33 per cent to 2.57 per cent in the highest age-group. The increase, occurring without evidence of tissue degeneration apparently is a normal process without causal relation to pathological calcification of the intima. This is

demonstrated clearly by a comparison of the calcium content of the media with the corresponding intima. In the middle age-group, for example, six medias contained approximately equal quantities of calcium. Two had no intimal changes, three had moderate atherosclerosis, and the other had marked atherosclerosis and calcification. The lack of correlation between intimal and medial calcification was more obvious in the highest age-group. Of the two aortas in which the media contained the lowest calcium content, the intima was markedly calcified. In one aorta, where the media had the highest calcium content, the intima had the slightest atherosclerosis, without calcification. A similar increase of cholesterol and of calcium with advancing age has been reported by Bürger (11) not only in the aortas of man, but also of horses and cattle.

Moisture. Dehydration of the tissue colloids in the media has been proposed as an important factor of atherosclerosis. This opinion is based upon the observations by Bürger and Schlomka (12) that tissues, such as costal cartilage, cornea and lens, lose water progressively with age. The loss in water, accordingly, decreases the capacity of the tissues to hold substances in solution, whereby the least soluble constituents, namely, cholesterol and calcium, precipitate. However, table 1 revealed no significant change in the moisture content of the media with increase in age. This agrees with the results reported by Bürger (11).

Summarizing the chemical changes of the media with age, there is a significant increase in the free and total cholesterol, phospholipids, galactosides and glycerides as the tissues become older. There is a marked increase in the calcium but no change in the moisture content. All of these changes have no obvious relationship to the severity of the intimal lesions. Chemical evidence, therefore, does not support the opinion that atherosclerosis occurs as a result of changes in the media.

Macroscopically and microscopically normal intima tissue is relatively rare; consequently too few analyses were carried out for conclusions regarding the effect of age on chemical changes in the intima. Tissues from five normal intimas were analyzed. In general, they had a higher lipid content and lower calcium content than the corresponding media tissue. The average values for intimal constituents calculated as per cent of dry tissue were: free cholesterol, 2.26 per cent; total cholesterol, 5.92; phospholipids, 2.65; galactosides, 0.98; total lipids, 14.4; and calcium 0.23.

CHANGES IN THE CHEMICAL COMPOSITION OF THE AORTA WITH ATHEROSCLEROSIS. *Cholesterol.* The first quantitative determinations of cholesterol in atherosclerotic aortas were reported by Windaus (77). He found in two normal aortas 0.119 and 0.107 per cent free cholesterol; and 0.032 and 0.047 per cent esterified cholesterol. In two atheromatous aortas, the values were 0.741 and 0.673 for the free cholesterol and 1.043 and 0.792 for ester cholesterol. Thus, the atheromatous tissues contained six to seven times as much free cholesterol and about twenty times as much esterified cholesterol as normal tissues.

The next significant contribution to the chemistry of atherosclerosis was by Schönheimer (59, 60), who made a systematic study of the effect of age and

atherosclerosis on the cholesterol content of the aorta. In the normal aortas of youths, he found only small quantities of lipids; with increasing age and increasing atherosclerosis there was a marked rise in the lipid content. In a series of these aortas the lipid content ranged from 0.2 to 1.8 grams. The proportion of cholesterol in the extract was relatively constant, varying only from 23 to 32 per cent of the total lipids despite the great variation in the amount of total lipids. The esterified cholesterol, however, increased with the rise in total lipids, amounting to 57 per cent of the total lipids in the atherosclerotic aortas. On the basis of these results, Schönheimer concluded that the original lipids of the aorta are augmented, in the development of atherosclerosis, by a lipid mixture containing a constant proportion of free and total cholesterol. Thus analytical evidence corroborated the view that the lipids of the blood infiltrate and are deposited in the intima.

Quantitative analyses of human aortas by Kimmelstiel (32) demonstrated a proportional increase of cholesterol, phosphatides and galactosides until atheromas formed. The relations then changed and cholesterol increased rapidly in comparison with the other lipids. Atherosclerotic aortas of non-diabetics according to Lehnherr (41) contained increased amounts of cholesterol, phospholipids and fatty acids; a greater proportion of total cholesterol in the lipid fraction; and larger quantities of calcium and phosphorus with a diminished Ca/P ratio. The lipid deposits in the atherosclerotic aortas of diabetics were greater, but the quantitative relations of the fractions were similar. The analyses of the "fat" content in 500 aortas by Rosenthal (57) confirmed those of previous reports disclosing a larger total lipid content in the tissues of aortas with atherosclerosis. He made no determinations of the individual lipids.

These analyses of entire aortas gave valuable information but did not differentiate between the chemical changes which occur with advancing age and those due to the disease. This was emphasized by Meeker and Jobling (45) who obtained a better understanding of the chemical changes occurring with the atherosclerosis of the aorta by analyzing the individual lesions, separated from the other tissue, and by comparing the values with those of the normal tissues.

In the normal tissues, 40 per cent of the lipids was cholesterol, of which 28.4 per cent was esterified and 12.6 per cent free; 16 per cent was phospholipids and about 20 per cent was unidentified. As the lesions increased in severity, the proportion of cholesterol increased greatly, the increase being due almost entirely to free cholesterol. The ratio of free to bound cholesterol increased from 0.43 in the normal tissue to 1.04 in the most advanced lesions. A later study, by Zeek (78), substantiated these results.

As a part of a chemical study of atherosclerosis in the human aorta, Weinhouse and Hirsch (71) separated atherosclerotic lesions of the intima from the surrounding tissues; determined the calcium, lipid and moisture content; and compared these values with those of the normal intima tissue. The atherosclerotic lesions were separated carefully from the media and surrounding intima. They were classified as fatty plaques, fibrous plaques, calcified plaques, and atheromatous ulcers. The first was the nodular or streaked yellow lesion in

which the lipid material was mainly intra-cellular, and the intima thickened slightly but not scarred appreciably. The second form was the raised nodule or diffuse plaque of tough fibrous tissue with a pearly luster, necrosis, and confluence of lipids. The third form consisted of brittle calcified plaques with soft necrotic centers. The atheromas were ruptured or about to rupture and contained, in addition to soft lipid material, blood clots and calcium deposits. The results of these analyses are summarized for comparison with those of normal intima in table 2.

They demonstrated a progressive increase in the lipid content of the diseased tissues. The relatively low lipid content of the calcified lesions was due to the great density of the calcium salts contained therein.

Cholesterol. As the lesions progressed, the proportion of the cholesterol in the lipid extract rose from 14.2 per cent in the normal intima to 27 per cent in the late stages. The proportion of cholesterol esters increased less rapidly and in

TABLE 2

Average values of constituents of intimal lesions

TYPE OF LESION	MOISTURE*	LIPID EXTRACT†	CHOLESTEROL‡		PHOSPHATIDE‡			FATTY ACIDS‡		
			Free	Ester	Ether soluble	Ether insoluble	Total	Galactoside	Neutral fat, etc.	Calcium†
Normal intima	71.6	14.4	14.2	38.6	13.7	6.4	20.1	8.0	19.1	0.23
Early fatty plaques...	67.5	25.9	16.2	38.5	10.8	8.2	19.0	5.8	20.5	0.86
Fibrous plaques.....	66.5	27.2	18.1	47.5	5.9	9.0	14.9	4.5	15.0	3.94
Calcified tissues.....	38.6	12.8	21.9	47.2	3.9	9.3	13.2	4.6	13.1	24.3
Atheromatous ulcers..	60.8	36.0	27.2	42.1	5.8	10.2	16.0	4.3	10.4	10.1

* The content is given as percentage of wet tissue.
† The content is given as percentage of dry tissue.
‡ The content is given as percentage of total lipid.

the lesions with the most extensive necrosis their amount was lower than in the intermediate stages. In agreement with Meeker and Jobling (45), the ratio of combined to free cholesterol declined in the advanced lesions of atherosclerosis, despite an increase in both substances.

Phospholipids. There was a slight decrease in the total phospholipids from about 20 per cent in the normal intima to about 13 per cent in the calcified lesions. The constitution of this fraction, however, changed greatly with advancing severity of the atherosclerosis. In the normal intima, there was approximately twice as much of the ether-soluble phospholipids (lecithin and cephalin) as ether-insoluble phospholipids; in the severely diseased tissues, the proportions were reversed.

Other lipids. Free and combined cholesterol and phospholipids together comprised from 60 to 80 per cent of the total lipids in atheromatous lesions. The remainder consisted of glycerides, fatty acids, and small, though measurable quantities of a reducing lipid substance, presumably galactoside.

Calcium. The normal intima contained much smaller amounts of calcium than the media. In five samples of such tissues, the calcium content averaged 0.23 per cent. An increase was observed even in the early plaques, and as the lesion developed, deposition of calcium proceeded rapidly even before visible hardening had occurred. In the grossly calcified lesions the calcium content varied from 17 to 37 per cent, indicating that almost complete replacement of the normal tissue structure by lipids and mineral matter had occurred.

FACTORS IN THE DEPOSITION OF LIPIDS. *Origin of the lipids.* Determinations of the composition of the lipids in the intima and in atherosclerotic lesions have added considerable support to the infiltration theory. If the deposits in atherosclerosis of the aorta arise by infiltration of the plasma lipids, the lipid composition of the fatty deposits, with little or no necrosis, should approximate the composition of the lipids in the blood plasma. The most comprehensive study of the lipid composition of human plasma is by Page, Kirk, Lewis, Thompson and Van Slyke (50). They determined free and total cholesterol and phospholipid in blood plasma of normal men of ages from 20 to 101 years. The analyses,

TABLE 3

*Comparison of lipid composition of blood plasma and arterial tissues**

	BLOOD PLASMA	INTIMA	EARLY PLAQUES	MEDIA
Free cholesterol	14.1	14.2	16.2	17.3
Cholesterol esters	38.3	38.6	38.5	16.7
Phospholipids	22.8	20.1	19.0	34.1
Neutral fat, etc.	23.3	27.1	26.3	31.9

* The values are given as percentage of total lipid.

made according to the gasometric methods of Van Slyke and co-workers, are the best figures available at present. As cerebrosides were not determined by these authors, this fraction has been included in the neutral fat fraction of the figures reported by Weinhouse and Hirsch (71) to provide a better basis of comparison between the two sets of values. Table 3 demonstrates a striking agreement between these analyses, despite the differences in procedures.

The close agreement in composition between lipid extracts from the plasma and the normal intima and the wide differences between the lipid extracts from the latter and the media indicate that the lipids of the intima originate in the plasma rather than in the protoplasm of the cells. The comparison also supports the validity of Anitschkow's opinion that the intima is freely permeable to the lipids of the plasma and that the intima has no selective action on these lipids. The close agreement between the composition of the extracts from the normal intima and that of the early fatty deposits suggests further that these deposits arise through non-specific deposition of the plasma lipids. The changes in lipid composition which occur in the late stages of the lesions may result from several processes. These are phagocytosis and other reactions against a foreign sub-

stance; necrosis resulting from altered nutrition; or chemical changes of the lipid substance deposited in the tissues. These processes, however, are secondary to the initial deposition of the lipids.

The lipids of the human blood. Page, Kirk, Lewis, Thompson and Van Slyke (50) observed no regular important changes in any lipid fraction of the blood from youth to old age. Their statistical analyses indicated no greater difference between age classes than would be expected from the variability with individuals of similar age. When the total lipids in the plasma increased, the free and ester cholesterol, the phosphatids and the neutral fats participated in this increase, but not to the same extent. Free cholesterol changed the least and neutral fat the most.

The concentration of cholesterol in the serum of normal human beings of both sexes averages about 200 mgm. with a range of from 100 to 400 mgm. per 100 cc. (70). Except for a pronounced rise during childhood, the cholesterol level remains constant. According to the reports of many investigators, during adult life the cholesterol level of the blood tends to remain constant and is not influenced by environmental conditions or living habits. Long periods of starvation or cholesterol deprivation on the one hand, or overfeeding with cholesterol on the other hand, produce little if any changes in the blood cholesterol. When great changes have been observed in normal individuals the amounts of cholesterol administered have been so large as to be unphysiologic. The changes in the blood lipids in alimentary lipemia are almost solely in the glyceride fraction which may vary widely under normal circumstances. Apparently in man, hypercholesteremia does not develop from the ingestion of moderate quantities of cholesterol.

The blood lipids in human atherosclerosis. The relationship between hyperlipemia and atherosclerosis in man is obscure. The influence of the amount and distribution of the blood lipids has been studied extensively, but the results are controversial. Since atherosclerosis develops without definite symptoms, determination of its presence and extent is difficult by physical examination. Therefore the relationship of blood lipid levels to this disease can be inferred only in studies of the blood lipids of patients with hypertension, angina pectoris and other diseases closely associated with atherosclerosis.

Mjassnikow (46) observed elevated levels of the blood cholesterol in angina pectoris, the values ranging from 190 to 440 mgm. per cent compared with 120 to 170 mgm. per cent for normal subjects. Davis, Stern and Lesnick (15) likewise reported blood lipid levels frequently elevated in patients with angina pectoris. About 60 per cent had total blood cholesterol levels over 250 mgm. per cent, whereas only 20 per cent of normal subjects had values this high. Values higher than normal for the blood phospholipids and fatty acids also were observed. In seventy-three patients with arteriosclerosis obliterans, unassociated with diabetes or hypothyroidism, Barker (7) found the mean value for blood cholesterol to be 263 mgm. per cent, compared with 218 mgm. for normal subjects. Poindexter and Bruger (54) observed average values of 250 mgm.

per cent for the blood cholesterol in twenty-four patients with arteriosclerosis and in nineteen with hypertension and arteriosclerosis, as compared with 195 mgm. per cent in a normal group.

Other investigators, however, have reported normal levels of the blood cholesterol in clinical diseases associated with arteriosclerosis. Andes, Kampmeier and Adams (1) observed no difference in the cholesterol level of the blood in normal and arteriosclerotic negroes. Page, Kirk and Van Slyke (51), in sixteen patients with hypertension uncomplicated by nephritis, found normal blood lipid levels. In no instance were the free and total cholesterol, phospholipids and total lipids outside the range of normal values; nor were the mean and standard deviations for these lipids different from those in normal subjects. Elliot and Nuzum (20) found no significant elevation of blood cholesterol in fifty-three patients with hypertension and concluded that there was no correlation between the severity of clinical arteriosclerosis and the level of the blood cholesterol.

Probably the best study is by Landé and Sperry (37). Recognizing the difficulties in measuring the extent of arteriosclerosis by clinical methods, they determined the blood cholesterol, postmortem, of persons dying suddenly from violence. After comparing these values with the degree of atherosclerosis, and eliminating those complicated by infection or organic disease, they concluded that no relationship existed between the blood cholesterol level and the severity of atherosclerosis.

Apparently, many if not most cases of atherosclerosis are unaccompanied by hypercholesteremia. Although the influence of the blood lipids in the development of this disease has not been disclosed, there seems to be no doubt that the disease may develop without obvious abnormality of the blood lipids.

In addition to the results of experimental atherosclerosis in rabbits to be discussed later, clinical evidence indicates that hyperlipemia is a factor in atherosclerosis. The close association between high blood cholesterol levels and atherosclerosis in diabetes has been discussed by Gibbs, Buckner and Bloor (22), White (76), Dragstedt, Clark, Julian and Vermeulen (17), and Rabinowitsch (55). The high incidence of atherosclerosis in systemic xanthomatosis, associated with hypercholesterolemia, has been reported by Thannhauser (63), Montgomery and Osterberg (47), and Müller (48). The atherosclerotic tendency among diabetics, according to Duff (18), may be due not necessarily to hypercholesteremia, but to other profound metabolic disturbances in this disease. This opinion receives support from the recent discovery of a pancreatic hormone, lipocaic, the absence of which causes the deposition of lipids in the liver (16, 17). Perhaps the deposition of lipids in atherosclerosis also may be the result of a hormonal deficiency, although experimental investigation of this possibility has yielded contradictory results. Thus, Huber, Brown and Casey (30) reported that lipocaic prevented the rise in the blood cholesterol and the development of atherosclerosis which otherwise occurred regularly in rabbits fed cholesterol. Dragstedt and his co-workers (66), however, were unable to confirm this. Data are also conflicting with regard to the influence of the lipotropic factor, choline, in experimental atherosclerosis. Himsworth (25) and Baumann and Rusch (8)

observed no effect, but Steiner (62) found that choline inhibited the formation of atherosclerotic lesions and hastened their removal. The presence or absence of such lipotropic factors may explain why the basic diet is important in the development of experimental cholesterol atherosclerosis.

Physical distribution of the lipids in the blood. Although particles of lipid from 0.5 to 1 micron diameter in the blood plasma, according to Bloor (10), are lipid in transport, the factors concerned with this physical distribution of the lipids in the blood plasma have not been considered in atherosclerosis. Much of the lipid absorbed from the intestine reaches the blood in this form and is removed and stored as fat. When the lipid particles accumulate in the blood, lipemia results. Ludlum, Taft and Nugent (42) believed from their studies of the tiny particles of lipid in the blood that the particles are stabilized by a protein film because the first zone of aggregation of the particles occurred at a pH range of 4.7 to 5.3, approximately the iso-electric points of albumin and globulin, and the droplets coalesced when the acidity was sufficient to precipitate the protein and destroy the film. The membrane about globules of fat in milk, according to Palmer and Wiese (52), is a mixture of protein and phospholipid. Churning of the milk removed the membrane. Hirsch and Weinhouse (29) injected emulsified cholesteryl oleate intravenously into rabbits to determine, if possible, its localization in the tissues. The particles of emulsified cholesteryl oleate ranged in size to considerably less than the diameter of erythrocytes. Although large amounts of the oleate were injected, the free and ester cholesterol content of the serum within a few minutes after an injection was not changed appreciably. The injected cholesteryl oleate, apparently, was removed rapidly from the blood. Histological examination demonstrated large amounts of particulate lipid material in the tissue phagocytes. The fat depots of a rabbit injected repeatedly with emulsified cholesteryl oleate stained with scarlet red were colored with the dye and the tissue phagocytes were laden with large quantities of the stained cholesteryl oleate. These experiments demonstrated that particles of lipid material are removed from the blood by tissue phagocytes like other particulate substances. Hirsch and Weinhouse (29) noted that where the fine emulsion of the cholesteryl oleate had broken and larger masses of the lipid had lodged in the tissues, phagocytosis was supplemented by growths of foreign body granulation tissues. The degree of dispersion of the lipid material, accordingly, seemed important in determining how the tissues utilized or disposed of the lipid material, a principle harmonious with tissue reactions toward particulate material of other chemical composition.

Site of the lipid deposits in the aorta. Opinions differ as to whether the lipid deposits of the intima are intracellular, primarily, and later extracellular with necrosis of the cells; or are extracellular at first, and then intracellular. Klotz (36) believed that the lipid material of the small fatty deposits in the intima of the aortas of the young at first is chiefly in large phagocytes. Later, the lipid droplets appeared outside of the cells, especially along the inner elastic fibers. Although Klotz considered the fat droplets free in the tissues as having been derived from disintegrated phagocytes, Wells (72) postulated that the fat drop-

lets, at first, may be free in the injured tissues and secondarily are phagocytized by the cells. Duguid (19), he stated, believed the latter more probable. Leary (40), thought the lipophages developed from the free mononuclear cells of the blood that migrated selectively into the intima. According to Wells (74), and others, the lipid deposits in the intima may be temporary and do not lead to atherosclerosis. McMeans and Klotz (43) observed a disappearance of lipid deposits in the aorta of rabbits with experimental atherosclerosis when the feeding of cholesterol was discontinued after the aortic lesions had developed. In animals and apparently in man (Wells) lipid deposits may cause local fibrous thickenings of the intima.

If we recognize that the lipids of the blood are transported in the form of small particles and that the normal intima and the simple lipid deposits of atherosclerosis have a lipid content corresponding in composition to the lipids of the blood, much of the reason for this controversy disappears. Lipids may be considered to be in particulate dispersion in the blood and interspaces of the intima tissues. Chemical analysis demonstrates this lipid material in the intima although no lipid is found by morphologic examination. An increase in the particle size of the lipids brings them into the range where phagocytosis occurs. This may be considered to be the initial stage of atherosclerosis.

Tissue reactions caused by lipids. The chemical analyses which demonstrated a close agreement in composition of the lipids of the blood, the normal intima, and the fatty plaques in atherosclerosis of the aorta indicate that a nonspecific infiltration of the intima by the plasma lipids occurs. Each lipid has a specific effect when deposited in tissues. The effects which the individual lipids, free and ester cholesterol, phosphatides, galactosides, fats, and their hydrolysis products, notably the fatty acids, cause in tissues must receive consideration in evaluating the significance of the fatty deposits in the subsequent changes of the intima. Cholesterol and cholesteryl oleate (28) deposited in the tissues stimulate a foreign body reaction; cerebrosides cause a pseudoxanthoma cell response; and lecithin, a polyblastic tissue reaction. With mixtures of the lipids, a differentiation on the basis of tissue reactions is impossible. This loss of characteristic tissue reactions with lipid mixtures led Kimmelstiel and Laas (33) to believe that the chemical character of lipid alone was not responsible for the tissue reactions. The fibroblastic tissue reaction with giant cells, they stated, was the response toward an insoluble substance. This reaction did not occur when cholesterol was mixed with lecithin and was highly dispersed. The types of xanthoma cell produced experimentally by such a mixture, they stated, depended upon the colloidal dispersion of the lipid and not on its chemical character.

The effect of lipid mixtures on arterial tissues was investigated in dogs by Christianson (13). The technical difficulties encountered in these experiments were considerable, and the lipid mixtures injected lodged usually in the media and not in the intima. The lipids, however, produced fibrous scars depending upon their composition. Those composed of glycerides and fatty acids caused marked inflammatory reactions and finally a scar; those composed of glycerides mixed with calcium soaps caused a chronic inflammation which also resulted in

a scar. Fibrous plaques formed in the intima over these lesions. Hagerty (23) reported that oleic acid introduced into the tissues of rabbits and dogs caused a severe necrosis and a marked growth of scar tissues. According to Hirsch (27) some of the factors involved in reactions of tissues about glycerides are the acidity developed in the surrounding tissues by their hydrolysis; the physical state of the fatty acid, whether solid or liquid; the solubility of the soaps formed and their chemical structure. Many of the fatty acids in tissues are non-specific irritants, stimulating mainly fibroblastic, monocytic, and epithelioid cells. Soaps of these fatty acids, relatively insoluble in the tissues, stimulated foreign body granulation tissues.

There is also much evidence in the many reports on the various forms of lipid pneumonias (53) and on the lesions of traumatic fat necrosis (26) that lipids cause marked inflammatory reactions and growths of fibrous scars in human tissues. Some of these tissue reactions develop rapidly, others evolve slowly.

According to the evidence presented, lipids deposited in tissues stimulate inflammatory reactions which eventually are reduced to fibrous plaques. The different lipids in these deposits contribute variably to the sum total of the tissue reactions and among them, the fatty acids seem to have the greatest irritative properties. The factors concerned with the stabilization of the lipid particles in the tissues, also, are important.

THE RELATION OF THE LIPIDS TO CALCIFICATION. The absence of observed abnormalities in the calcium content of the blood suggests that local tissue factors are responsible for the calcification in atherosclerosis of the vessels. The frequent occurrence of calcification with atherosclerosis and with certain forms of fatty changes led to the opinion that calcium deposition in some way was connected with the presence of lipids. This hypothesis was emphasized by Klotz (34, 35) who claimed to have observed the presence of calcium salts of fatty acids in degenerating tissues; and to have observed infiltration of calcium into fats or fatty acids imbedded in tissues of the body. These conclusions were based on microscopic observations alone, and though often quoted, this theory has not been confirmed. Aschoff (4) suggested that the formation of calcium soaps preceding calcification occurs through splitting of cholesterol esters, whereby the fatty acids liberated combine with calcium ions to form calcium soaps and the fatty acid is replaced gradually by phosphate and carbonate ions. If Aschoff's theory were correct, the ratio of cholesterol esters to cholesterol should decrease with gradual evolution of the atherosclerotic lesions. In an investigation of this, Schönheimer (61) observed no change in the ratio and concluded therefore that Aschoff's theory was incorrect. Subsequently, however, Weinhouse and Hirsch (71) and Meeker and Jobling (45) observed a lowered cholesterol-ester to cholesterol ratio in calcified atherosclerotic lesions.

As Hirsch (26, 27) has pointed out, chemical combination between positive ions in an aqueous phase and negative ions in the lipid phase may account for the occurrence of chemical reactions between the two systems. In support of this view are the observations of Langmuir and Schaefer (38), who found that fatty acids in the form of monomolecular films floating on solutions of calcium

and barium salts, were converted to the corresponding soaps to varying extents, depending upon the pH of the aqueous medium. Similar results were reported by Hartsuch (24), who found that magnesium ions were lost from a dilute aqueous solution of magnesium phosphate when shaken with oleic acid. The transfer of magnesium ions was appreciable at a pH of 7. There is, however, much other evidence refuting Aschoff's hypothesis, mainly obtained by Wells (74) and discussed by him at length. Briefly summarized, the evidence against the idea that calcification takes place through intermediate reaction with lipids is as follows: 1, calcium soaps have never been isolated from calcified aortas or from any other calcified pathological process. This fact has been demonstrated by Baldauf (6), Wells (73), and in unpublished experiments by Weinhouse and Hirsch; 2, even if calcium soaps were present in the body, calcification may not occur, for Wells (75) has introduced calcium soaps into living tissues and when removed they had not been converted into inorganic salts; and 3, the composition of the calcified portion of atherosclerotic aortas closely resembles that of bone. Accordingly, the mechanism of calcification with atherosclerosis is similar to that with bone formation. The analyses, reviewed by Wells (74), agree that the calcium in the lesions of atherosclerosis is mainly in the form of calcium phosphate, and small, variable amounts of carbonate and magnesium salts. The analogy to bone formation is emphasized by the development of bone tissues in old lesions. The similarity of calcified intimal lesions to true bone was demonstrated by Mr. Raymond Pepinsky of the Physics Department of the University of Chicago, who at our request investigated the x-ray diffraction patterns of several calcified atheromatous lesions. These patterns were identical with those given by minerals of the apatite group and were identical with the diffraction patterns of bone observed by Taylor and Sheard (64). According to these authors the inorganic portion of bone is composed of the apatite, $3Ca_3(PO_4)_2 \cdot CaCO_3$.

Chemical and physical evidence favor the opinion that calcification with atherosclerosis is a secondary process similar to the calcification in other tissues in the body where necrosis has occurred.

THE COMPOSITION OF THE BLOOD AND TISSUE LIPIDS IN EXPERIMENTAL ATHEROSCLEROSIS. The lesions of the aorta and other tissues in the experimental form of atherosclerosis produced in animals by diets containing added amounts of cholesterol have been described fully by Anitschkow (3), Duff (18) and Leary (39, 40). The similarity between the human and experimental forms of atherosclerosis has been emphasized regardless of certain obvious differences. Atherosclerosis develops in rabbits only after a significant hypercholesteremia has been present. Hypercholesteremia has not been observed usually in human atherosclerosis. The deposition of the lipids in the wall of the aorta and arteries in the experimental form of atherosclerosis is part of a systemic lipid infiltration of the tissues.

Blood. A daily intake of cholesterol of less than one per cent of the diet in rabbits produces a significant hypercholesteremia in a week or less, and in several months the blood cholesterol may reach high levels. Values over 2500 mgm.

per 100 cc. of serum have been observed frequently. The first observations of hypercholesteremia were recorded by Wacker and Hueck (69), who reported a twenty-fold increase in both free and total cholesterol of the blood after several months of the cholesterol diet. These observations were confirmed by Versé (67), who noted that the cholesterol levels of the blood increased more rapidly and with smaller daily feedings when the cholesterol was given dissolved in oil. The most extensive studies of the blood lipid changes in experimental atherosclerosis were reported by Page and Bernhard (49) and Weinhouse and Hirsch (71). These studies established that in addition to the cholesterol, there is a marked increase in the other lipids, notably the phospholipids and the glycerides. A comparison of the results of both analyses is in table 4.

At the height of lipemia there was an average increase of twenty-five to thirty-fold in the free and total cholesterol. Contrary to earlier reports there was no significant increase in the proportion of cholesterol esters. Despite the marked increase in total cholesterol the average ratio of free to total cholesterol, 0.28

TABLE 4

Blood lipid levels at height of lipemia

INVESTIGATOR‡	FREE CHOLESTEROL	TOTAL	ESTERS	PHOSPHOLIPID	GLYCERIDES	TOTAL LIPIDS
Page and Bernhard*.....	356	1435	1823	526	270	2902
Weinhouse and Hirsch†.....	452	1652	2010	706	718	3660

* Cholesterol administered as a solution in oil, 0.2 gram per day. Determinations made on heparinized plasma.

† Cholesterol administered without added fat, 1.0 gram per day. Determinations made on serum.

‡ Dauber and Katz (14) recently reported similar results with experimental atherosclerosis in chickens.

at the height of lipemia, was the same as in the normal rabbits. Phospholipids increased to seven times the normal value, whereas glycerides increased to five times normal. The total lipids were, on the average, eleven times the normal value. The increase in the blood lipids began in a few days, reached a maximum usually between fifty and one-hundred days on the cholesterol diet, and thereafter the amount declined. In animals surviving more than one hundred and fifty days under the conditions of the experiment, the blood lipids approached normal values. This decrease does not indicate development of a tolerance for cholesterol in these animals, because at this stage they lost weight rapidly and exhibited all the signs of starvation as though the normal absorption of food across the intestinal wall was interrupted. The tissues of the stomach and bowel of these rabbits had marked intracellular infiltrations of lipids.

The effect of added fats to the diet in facilitating the absorption of cholesterol and in increasing the lipemia is illustrated also in table 4. At the height of lipemia, both studies exhibit about the same lipid levels, although Page and Bernhard fed their rabbits only one-fifth the quantity of cholesterol. They adminis-

tered it dissolved in oil. These analyses of the lipids of the blood in experimental atherosclerosis demonstrated clearly that not only is the cholesterol content increased to high levels, but also all of the other lipids. In studies of the physical state of the blood lipids in experimental hyperlipemia in rabbits, Remesow (56) suggested that the increase in the lipids other than cholesterol may be due to a destruction or binding of the serum "lipases" by the excess cholesterol, thus causing the accumulation of lipids. This opinion is based on the inhibition of lipases by alcohols in general. However, the effect of excess cholesterol in causing increases in other blood lipids may not be due to any chemical effect of this sterol, but to its effect on the physical state of the blood lipids. The increase in hydrophobic components of the blood lipids, such as cholesterol and its esters, presumably could decrease the stability of the lipid complex, leading to a decrease in the dispersion of the colloid. Agglomeration into larger particles would follow and a marked decrease in the surface area exposed to enzyme activity would result.

Tissues. Weinhouse and Hirsch (72) found, in rabbits fed cholesterol, marked increases in the lipids of the viscera as well as of the aorta. The greatest changes were in the aorta, liver, and suprarenal glands. The lungs, kidneys, and spleen occasionally had lipid infiltration and correspondingly high cholesterol or cholesterol ester values, but usually there were only minimal changes in lipid content. The atherosclerotic aortas had a marked increase in free and total cholesterol as well as a significant though variable increase in the phospholipids and glycerides. The total lipids were, in general, increased two or three times above the level of those in the normal aortas. The livers of all rabbits fed cholesterol contained quantities of lipids substantially above the levels in control rabbits, the increase being greatest in the cholesterol ester fractions and least in the glycerides. The cholesterol content of the suprarenal glands was markedly increased. The free cholesterol was increased from two or three times above the content of normal tissues, and the cholesterol esters, which in the control analyses averaged 14 per cent, were doubled in the cholesterol-fed rabbits. These analyses of the suprarenal gland agree with those of Kay and Whitehead (31).

The significance of the hyperlipemia in experimental atherosclerosis. The relationship between hypercholesterolemia and atherosclerosis in the rabbit seems to be definite. The lesions of experimental atherosclerosis in rabbits, which closely resemble those of the human form, have not been produced in any other way. Despite variations in diet and superimposed types of mechanical and chemical injury, an excess of cholesterol in the blood appears to be necessary in the formation of the lipid plaques of the aorta and arteries. The evidence implicating cholesterol as a primary agent in the development of atherosclerosis has been presented forcefully by Leary (39). According to his views, the differences between human and experimental atherosclerosis may be attributed to differences in reaction of youthful and aged tissues to the presence of cholesterol and not to any fundamental difference in etiology.

In the experiments of Weinhouse and Hirsch (72), though hypercholesterolemia always preceded deposition of cholesterol, there was no close relationship between

the height or duration of the hypercholesteremia and the severity of the atherosclerosis. For example, of two rabbits fed the same daily amounts of cholesterol for the same period of time, the serum cholesterol of one reached a maximum of 2200 mgm. per cent; the other, 1500 mgm. per cent. The severity of atherosclerosis as determined by gross examination, and the amount of lipids deposited, were about the same. In two other rabbits with the same degree of hypercholesterolemia, one had severe, and the other had only a mild atherosclerosis. These results logically suggest that tissue factors play an important part in the development of experimental atherosclerosis.

TISSUE FACTORS IN ATHEROSCLEROSIS. The results of these chemical analyses indicate that tissue factors are important in the development of atherosclerosis in man and experimentally in rabbits. Weinhouse and Hirsch (72) did not find a close correlation between the height and duration of lipemia and the severity of the experimental atherosclerosis. These individual variations were found also in different viscera of the same rabbit, some visceral tissues being markedly infiltrated by lipids and others relatively unchanged.

The importance of tissue factors in the development of experimental atherosclerosis is indicated also by studies on the effects of iodine and thyroid hormone. According to Page and Bernhard (49), and Turner and Bidwell (65) the addition of iodine or thyroid extract to the diet prevents or inhibits the development of atherosclerosis in rabbits fed cholesterol, although these substances did not prevent or delay the hyperlipemia.

Since the atherosclerotic lesions are not distributed diffusely through the vessel wall, but are sharply localized, certain places in the arteries are more susceptible to lipid infiltration than others. The lesions tend to be more numerous in tissues subject to unusual mechanical strain or at sites with variations in tissue structure as, for example, along the posterior wall of the descending aorta, at the ostiums of arterial branches and at the bifurcation of the iliac branches. This suggests that conditions increasing the mechanical stress, such as hypertension, contribute to the development of atherosclerosis. As the cause and effect relationships are not clear, probably a large variety of conditions are concerned with this localization of the lesions. If hypercholesteremia alone played an important rôle in human atherosclerosis there should be a closer relationship between the disease and the blood lipids than has been demonstrated. Although hyperlipemia may favor the development of atherosclerosis, factors in the tissues are concerned with the actual lipid deposition.

MECHANISM OF LIPID DEPOSITION. The significance of the lipid deposits in the intima with atherosclerosis is disputed. Opinions differ as to whether the lipids at first are intracellular and with destruction of the cells become extracellular or at first are extracellular and secondly become intracellular.

Chemical analyses by Weinhouse and Hirsch (71), revealing a close agreement in the composition of the blood plasma lipids and that of the normal intima tissue, suggest that the intercellular spaces of the intima are filled with blood plasma. Accordingly, reactions which take place in the blood would be expected to occur also in the intima. The blood lipids of the plasma exist as colloidal complexes, ordinarily too small to be seen microscopically. In hyperlipemia,

however, the lipid complex agglomerates into larger particles, which are visible under the microscope and which give to such serums an opalescence. It is reasonable to conclude, therefore, that the phagocytic reactions observed in the intima occur as a response to the presence of coarsely dispersed lipids in varying stages of agglomeration. The primary cause of the precipitation of lipid in cholesterol-fed rabbits is probably the hyperlipemia; in human atherosclerosis it is mainly a tissue factor increased under conditions of hyperlipemia. Regardless of the cause of the precipitation of lipids, however, all the subsequent chemical processes and tissue reactions may be regarded as sequential to the initial separation of lipid in grossly dispersed particulate form.

SUMMARY

Chemical, physical and morphologic changes occur in arteries with age. These changes seem to be independent of atherosclerosis which is primarily a disease of the intima. The simple fatty deposits with atherosclerosis have the same lipid composition as the blood plasma and the normal intima. The identical composition of these tissue lipids strongly supports the view that the lipid deposits in atherosclerosis result from a non-selective deposition of the plasma lipids. Although cholesterolemia may favor the development of atherosclerosis, the lesions of this vascular disorder develop in adult life without an appreciable elevation of the blood cholesterol or obvious abnormality of the blood lipids. This suggests that factors in the tissues of the blood vessels leading to the deposition of lipids in the intima are important in the causation of the disease. The tissue factors concerned with the localization of the lipid deposits in the intima are not known. However, after the lipids are deposited and remain in the tissues in coarse particulate form, the subsequent stages of atherosclerosis follow. The evolution of the lesions from the simple fatty deposits to calcified atheromatous ulcers occurs because of several processes; among which are phagocytosis, physical or chemical effects on the tissues by the lipids or their decomposition products, disturbances in nutrition of the tissues, admixtures of blood, and reactions of the tissues against the lipids deposited. The calcification which occurs late in the evolution of the lesions probably is the same as that which develops in any necrotic or devitalized tissue of the body. The evidence presented in this review favors the conclusion that atherosclerosis develops from disturbances in the metabolism of lipids infiltrated into the tissues of the intima. The elucidation of the nature of the disturbances, however, depends upon further fundamental studies on the interactions and interconversions of the lipids in the blood and tissues.

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THE NATURE OF THE FORCES BETWEEN ANTIGEN AND ANTIBODY AND OF THE PRECIPITATION REACTION

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In one of his lectures on immunochemistry at the University of California in the summer of 1904 Svante Arrhenius said (1) that Ehrlich and other investigators, because of incomplete knowledge of the phenomenon of chemical equilibrium, had been led to invent artificial hypotheses in order to explain their observations in the field of immunology. Since that time, and especially during the last few years, workers in this field have made greater and greater use of the concepts and methods of physical chemistry, and in consequence many previously puzzling observations have been reasonably interpreted.

Another branch of chemistry which is of importance to immunology is modern structural chemistry, which deals with the detailed structure of molecules and with the nature of interatomic and intermolecular interactions (2). Our present knowledge of this subject, in large part won during the past dozen years, is now so firmly founded and so extensive that it can be confidently used as the basis for a more penetrating interpretation of immunological observations than would be provided by the observations alone.

In this paper we present, after a brief historical introduction, a discussion of the nature of the specific forces between antigen and antibody and of the precipitation reaction from the point of view of modern chemistry. Only the simpler aspects of the phenomena are discussed; such complicating factors as the rôles of complement, lipids, etc., in the reactions are disregarded in our discussion.

The history of the precipitation reaction began in 1897, when Rudolf Kraus (3) reported the results of his work with anticholera and antityphoid sera. His observations were soon verified and extended by Nicolle, Tchistovich, Bordet, Myers and other workers, who prepared precipitating antisera against a great number of antigens of varied nature. We shall not review this early work here, nor the later studies of the methods of preparing antisera and carrying out the precipitation reaction, since these topics and others dealing with special phases of the reaction have been very well covered in earlier reviews (4, 5, 6).

Two most important advances in the attack on the problem of the nature of immunological reactions were the discovery that the specific precipitate contains both antigen and antibody (7) and the discovery that antibodies, which give antisera their characteristic properties, are proteins. The verification of these facts was provided by the work of many investigators over a score of years. This work, which is summarized in Marrack's monograph (6, chap. II), culminated in the preparation of purified antibody by Felton and Bailey (8), Heidelberger and collaborators (9), and others, and the determination of its properties, including amino-acid composition and molecular weight, which show that it is very closely related to normal serum globulin (6, chap. II).

The work of Landsteiner (10) and other investigators on artificial conjugated antigens provided a great body of qualitative information on the specificity of antibodies, which, together with the experimental results for natural antigens, led to the independent proposal by Breinl and Haurowitz (11), Alexander (12), and Mudd (13) in 1930–32 of the theory of structural complementariness of antigen and specific antibody. The framework theory of precipitation was then developed by Marrack (6) and Heidelberger (14). These and other theories are discussed in some detail in the following sections of this paper.

A new period in the study of the precipitation reaction was initiated by the careful quantitative studies of Heidelberger and his collaborators (15) who determined the amounts of antibody and antigen in precipitates, and the similar work of Haurowitz (16) and others. Very recently, in order to test certain aspects of his detailed theory of the structure of antibodies (17), Pauling and his collaborators have carried out many quantitative experiments on the precipitation of antisera by polyhaptenic simple substances (18, 19, 20), a phenomenon first observed by Landsteiner and Van der Scheer (21).

THE NATURE OF THE SPECIFIC FORCES BETWEEN ANTIGEN MOLECULES AND ANTIBODY MOLECULES. The detailed information which has been gathered in recent years regarding the nature of the chemical bonds which hold atoms together into stable molecules has been summarized in monographs (2, 22). Instead of interacting strongly with one another, with interaction energy of 20 kilocalories per mole or more, to produce a chemical bond, two atoms may interact more weakly. The nature of these weak interactions is now well understood, and a brief discussion of it is given in the following paragraphs. The properties of antigen-antibody systems, especially the reversibility of complex formation, are such as to indicate that the antigen-antibody attraction is due to these weaker interactions and not to the formation of ordinary chemical bonds.

The weak interactions between two molecules may be classified as electronic van der Waals attraction, Coulomb attraction, attraction of electric dipoles or multipoles, hydrogen-bond formation, etc. The forces increase rapidly in magnitude as the molecules approach one another more and more closely, and the attraction between the molecules reaches its maximum when the molecules are as close together as they can come. The molecular property which determines the distance of closest approach of two molecules is the electronic spatial extension of the atoms in the molecules. It is possible to assign to each atom a *van der Waals radius*, which describes its effective size with respect to intermolecular interactions. These radii vary in value from 1.2 Å for hydrogen through 1.4–1.6 Å for light atoms (fluorine, oxygen, nitrogen, carbon) to 1.8–2.2 Å for heavy atoms (chlorine, sulfur, bromine, iodine, etc.). The shape of a molecule can be predicted by locating the atoms within the molecule with use of bond distances and bond angles and then circumscribing about each atom a spherical surface corresponding to its van der Waals radius. This shape determines the ways in which the molecule can be packed together with other molecules (2, sec. 24).

The most general force of intermolecular attraction, which operates between

every pair of molecules, is *electronic van der Waals attraction.* This type of electronic interaction between molecules was first recognized by London (23). A molecule (of methane, for example) which has no permanent average electric dipole moment may have an instantaneous electric dipole moment, as the center of charge of the electrons, in their rapid motion in the molecule, swings to one side or the other of the center of charge of the nuclei. This instantaneous dipole moment produces an instantaneous electric field, by which any other molecule in the neighborhood would be polarized; the electrons of the second molecule would move relative to its nuclei in such a way as to give rise to a force of attraction toward the first molecule.

This electronic van der Waals attraction operates between every atom in a molecule and every atom in other molecules in the near neighborhood. The force increases very rapidly with decreasing interatomic distance, being inversely proportional to the seventh power of the interatomic distance. Hence the electronic van der Waals attraction between two molecules in contact is due practically entirely to interactions of pairs of atoms (in the two molecules) which are themselves in contact; and the magnitude of the attraction is determined by the number of pairs of atoms which can be brought into contact. In consequence, two molecules which can bring large portions of their surfaces into close-fitting juxtaposition will in general show much stronger mutual attraction than two molecules with less extensive complementariness of surface topography.

Other types of molecular interactions result from the possession of a permanent electric charge, electric dipole moment, or electric moment of higher order by one or both of the interacting molecules. The effects of these charges and moments have been classified in various ways, as ion-ion forces, dipole-dipole forces, forces of electronic polarization of one molecule in the dipole field of another, etc. All electrostatic interactions are very much smaller in water than in a medium of low dielectric constant, and it can be shown by calculation, making use of known values of the effective dielectric constant of water for charges a given distance apart (24), that in general these electric forces are of minor importance, except when an isolated or essentially isolated electric charge is involved. The electrostatic attraction of a positive group such as a substituted ammonium ion and a negative group such as a carboxyl ion becomes significantly strong, with bond energy 5 kilocalories per mole or more, if the structure of the molecules containing the groups is such that they can come into juxtaposition.

A type of intermolecular attractive force which ranks in importance with the electronic van der Waals attraction and the attraction of oppositely charged groups is that associated with the structural feature called the *hydrogen bond.* The importance and generality of occurrence of the hydrogen bond were first pointed out in 1920 by Latimer and Rodebush (25) and summaries of the properties of the bond are given in the monographs quoted above. A hydrogen bond results from the attraction of a hydrogen atom attached to one electronegative atom for an unshared electron pair of another electronegative atom. The strength of a hydrogen bond depends on the electronegativity of the two atoms which are bonded together by hydrogen; fluorine, oxygen, and nitrogen, the

most electronegative of all atoms, are the atoms which form the strongest hydrogen bonds. The energy of a hydrogen bond between two of these atoms is of the order of magnitude of 5 kcal. per mole. This is so large as to have a very important effect on the intermolecular interactions of molecules capable of forming hydrogen bonds and on the properties of the substances consisting of these molecules.

In synthesizing our knowledge of intermolecular forces and of immunological phenomena into a definite picture of the antigen-antibody bond the immunological property of greatest significance is the specificity of the combining power of antibody for the immunizing antigen.

The forces of van der Waals attraction, hydrogen-bond formation, and interaction of electrically charged groups are in themselves not specific; each atom of a molecule attracts every other atom of another molecule by van der Waals attraction, each hydrogen atom attached to an electronegative atom attracts every other electronegative atom with an unshared electron pair which comes near it, and each electrically charged group attracts every other oppositely charged group in its neighborhood. The van der Waals repulsive forces which determine the van der Waals radii of atoms also are not specific; each atom in a molecule repels every other atom of another molecule, holding it at a distance corresponding to the sum of the pertinent van der Waals radii. We see, however, that specificity can arise in the interaction of large molecules as a result of the shapes of the molecules. Two large molecules may have such spatial configurations that the surface of one cannot be brought into contact with the surface of the other except at a few isolated points. In such a case the total electronic van der Waals attraction between the two molecules would be small, because only the pairs of atoms near these few isolated points of contact would contribute appreciably to this interaction, and, moreover, the distribution of hydrogen-bond forming groups and of positively and negatively charged groups of two molecules might be such that only a small fraction of these groups could be brought into effective interaction with one another for any position and orientation of one molecule with respect to the other; the energy of attraction of these two molecules would then be small. If, on the other hand, the two molecules possessed such mutually complementary configurations that the surface of one conformed closely to the surface of the other, if, moreover, the electrically charged groups of one molecule and those of the other were so located that oppositely charged groups were brought close together as the molecules came into conformation with one another, and if the hydrogen-bond forming groups were also so placed as to form the maximum number of hydrogen bonds, the total energy of interaction would be very great, and the two molecules would attract one another very strongly. We see that this strong attraction might be highly specific in the case of large molecules which could bring large areas of their surfaces into close contact. A molecule would hence show strong attraction for another molecule which possessed complete complementariness in surface configuration and distribution of active electrically charged and hydrogen-bond forming groups, somewhat weaker attraction for those molecules

with approximate but not complete complementariness to it, and only very weak attraction for all other molecules.

This specificity through complementariness of structure of the two interacting molecules would be more or less complete, depending on the greater or smaller surface area of the two molecules involved in the interaction. It may be emphasized that this explanation of specificity as due to a complementariness in structure which permits non-specific intermolecular forces to come into fuller operation than would be possible for non-complementary structures is the only explanation which the present knowledge of molecular structure and intermolecular forces provides.

This theory of structural complementariness of antigen and antibody was first suggested, in less detailed form than above, by Breinl and Haurowitz (11), Alexander (12), and Mudd (13). A detailed discussion of the structure of antibodies and of a postulated method of their formation has been presented by Pauling (17), who has also reviewed the evidence supporting the theory of complementariness.

It was suggested by Breinl and Haurowitz and by Mudd that the effect of an antigen in determining the structure of an antibody might involve the ordering of the amino-acid residues in the polypeptide chains in a way different from that in the normal globulin. Rothen and Landsteiner (26) then pointed out that the possibility of different ways of folding the same polypeptide chain is worth considering, and this postulate was amplified by Pauling (17), who assumed that all antibody molecules contain the same polypeptide chains as normal globulin, and differ from normal globulin only in the configuration of the chains. This assumption was made because it permits the formulation of a simple proposed mechanism of manufacture of specific antibodies. An antibody molecule, capable of existing in any one of a great number of configurations with nearly the same energy, is synthesized, except for the final folding step, in the same way as normal globulin. If no foreign substance is present, the chain then folds into a stable configuration, characteristic of normal globulin; but if an antigen molecule is present, the chain folds into a configuration stable in the presence of the antigen, that is, into a configuration complementary to that of a portion of the surface of the antigen molecule. This explanation of the ability of an animal to form antibodies with considerable specificity for an apparently unlimited number of different antigens (27), as shown especially by the work of Landsteiner (10), is compatible with the principles of structural chemistry and thermodynamics as well as with the immunological evidence.

To illustrate the way in which the complementariness theory accounts for many reported observations we shall mention only one point, taken from the great body of results on azoproteins obtained by Landsteiner. He observed a pronounced cross reaction between an azoprotein made from *m*-aminobenzoic acid and an antiserum to an azoprotein made from 4-chloro-3-aminobenzoic acid and a different protein, but no reaction with the haptenic groups reversed. The explanation of this is that the antibody to the 3-azo-4-chlorobenzoic acid group conforms closely to this haptenic group, allowing either this group or the

3-azobenzoic acid group, which differs in the replacement of the chlorine atom by a smaller atom, hydrogen, to fit into the complementary cavity in the antibody; but the 3-azo-4-chlorobenzoic acid group cannot fit into a cavity designed for the smaller haptenic group, and so the reverse cross reaction does not occur. In a quantitative extension of Landsteiner's work on hapten inhibition of precipitation reactions of simple polyhaptenic substances (10), Pauling and collaborators (28) have recently reported a great deal of evidence in support of the complementariness theory. They interpreted their results on the inhibition of the precipitation reaction between dyes containing *p*-azophenylarsonic acid groups and antisera to hapten-homologous azoproteins to obtain numerical values of the strength of the bonds formed by these antibodies with over twenty-five different haptens. The observed correlation between the bond strengths and the structure of the haptens is that which would be expected from the complementariness theory.

This theory is not greatly different from some earlier proposals, such as Ehrlich's lock-and-key analogy (29), but it differs greatly from others. For example, Buchner (30) considered that antigen molecules are split up and incorporated into the antibody molecules, thus imparting specificity to them. This theory or a closely related theory has been supported by many people, including Burnet (31), who proposed a mechanism for the manufacture of antibodies in the image of the antigens: the antigens act as templates for the manufacture by the body of specific enzymes, which then serve as the molds for the production of antibodies similar to the original antigens. Until recently there has been no suggestion as to why antibodies similar in structure to an antigen should combine specifically with it. Recently, however, Jordan (32) has stated that a strong attraction would occur between such identical or nearly identical molecules because of the quantum-mechanical resonance phenomenon; this has been denied by Pauling and Delbrück (33), who pointed out that the resonance energy would be so small as to be ineffective. Chemical evidence against the identity of antibodies and specific antigens has been presented by many authors, of whom the most recent are Haurowitz, Vardar, and Schwerin (34).

Forty years ago there was under way a keen controversy between Ehrlich and Bordet, and their respective supporters, as to whether the bonds between antibodies and antigens are chemical bonds or are physical forces of the sort producing surface phenomena such as adsorption. The modern point of view resolves this argument, but not in favor of either side; in fact, as in recent years an understanding has been obtained of the forces responsible for surface phenomena it has been found that these forces are the same as those which are operative in chemical reactions, so that the old distinction between chemical and physical forces has lost most of its meaning.

THE NATURE OF THE PRECIPITATE. Under suitable conditions (salt concentration, antibody-antigen ratio, etc.) the first stage of combination of antibody and antigen, which may make itself evident in change in toxicity or other properties of the antigen, is followed by precipitation. There has been much discussion as to whether or not this second stage is specific, like the first stage,

or is non-specific. Direct experimental evidence on this point, while not conclusive, favors the view that the reaction is specific. The most pertinent observations are those on the agglutination of mixed cellular antigens by mixed antisera; Topley and collaborators (35) noted the formation of separate clumps by the different cells, whereas Abramson (36) observed mixed clumping. Hooker and Boyd (37) found that mixed human and chicken erythrocytes gave separate clumps under some conditions and mixed clumps under other conditions. The fact that separate clumping is observed at all strongly favors the concept of a specific second stage, since mixed clumping might result from mechanical intertwining of specific clumps, whereas separate clumping would hardly be expected to result from non-specific interaction. Heidelberger has pointed out that in those cases where mixed clumping takes place the cells used were either very large or of greatly different sizes (38).

A reasonable theory of agglutination and precipitation, the framework theory (lattice theory[1]), was proposed in 1934 by Marrack (6), and has received strong support from the theoretical considerations and experiments of Heidelberger (9, 14, 15) and Pauling (18, 19, 20, 28) and their collaborators.

It is clear that, after we have accepted a mechanism for the specific attachment of antibody molecules to a cellular antigen, the simplest possible explanation of the agglutination of the cells is that it results from the same mechanism; if an antibody molecule had the power of specific attachment to two cells, it could form specific bonds with the two cells and thus hold them together, and the repetition of this process would lead to the formation of larger and larger clumps. Specific precipitation of antibodies and molecular antigens would result from the same mechanism if both antibody molecules and antigen molecules were multivalent (capable of forming two or more antigen-antibody bonds); larger and larger complexes A—B, A—B—A, A—B—A—B, etc., would form until the aggregates became macroscopic in size. The evidence supporting the framework theory has been reviewed by Marrack (6), Heidelberger (14), and Pauling (17); some of it, including that provided by recent work, is presented in the following section in connection with a discussion of the valence of antibody molecules.

The first of the theories of non-specific precipitation is the theory of neutralization of electrical charges. This theory was supported by many early investigators, who were attracted by the analogy with the well-known phenomenon of the mutual precipitation of oppositely charged colloids. Teague and Field (39) investigated the charges of agglutinins and bacteria and concluded that the former are positively and the latter negatively charged; it is now known, however, as the result of the application of improved experimental methods, that under ordinary conditions (normal hydrogen-ion and salt concentrations) antibody molecules and most antigens are negatively charged, and the theory of neutralization has in consequence been abandoned.

(The failure of precipitation or agglutination to occur in antigen-antibody

[1] We have adopted the name "framework theory" instead of "lattice theory" because of our belief that the framework of antibody-antigen precipitates does not usually have the regularity of structure which would be indicated by use of the latter expression.

systems with low salt concentration is, indeed, attributed to the electrostatic repulsion of the negatively-charged complexes in solution, which prevents the formation of large aggregates; agglutination or precipitation may occur in the presence of salt, the cations of which neutralize the negative charges of the complexes.)

Another theory of non-specific precipitation which was proposed soon after the discovery of the precipitation reaction is that the reaction results from the formation of a hydrophobic colloid, which precipitates in the presence of electrolytes. This theory has been revived recently by Eagle (40) and by Hooker and Boyd (37). Eagle's suggestion that the polar groups of the antigen which are assumed to be responsible for its solubility are masked by a layer of antibody molecules, which themselves turn their polar groups inward and present only non-polar groups toward the solvent, has been discussed by Marrack (6), who has marshalled some arguments against it. An important argument is that particles can be agglutinated by an amount of antibody very much smaller than the amount required to coat their surface; the most recently reported experiments of this sort (41) indicate that azoerythrocytes can be agglutinated by less than 0.02 per cent as much antibody as would cover their surface with a layer 3.5 Å thick.

Hooker and Boyd (37) have presented several arguments in support of the thesis that "... particles grow to visible size by the indiscriminate and non-specific accretion of other related or unrelated, small or large, aggregates whose primary nuclei are molecules or particles of antigen coated with antibody-globulin." As additional evidence for this concept and against the framework theory Boyd and Hooker (42) reported their failure to inhibit the agglutination of erythrocytes by use of an excess of hemagglutinin. In our opinion the fact that inhibition of agglutination of particles (43) as well as of precipitation of molecular antigens (44) by excess antibody has been observed gives strong support to the framework theory. The failure of inhibition to occur under ordinary circumstances may be due to the difficulty in saturating the multivalent antigens, especially cellular antigens with thousands of combining groups, as is indicated by the theories of Hershey (45) and Pauling (17). In particular, the experiments of Heidelberger and Kabat (43) on the agglutination by untreated pneumococci of pneumococci coated with antibody and then thoroughly washed are most easily explained by the framework theory. Hooker and Boyd (46, 47) have recently proposed the theory that precipitation of antibody by polyhaptenic dyes may result from the action of the dye molecules in pulling the antibody molecules to which they are bonded so tightly together as to prevent the solvent from reaching the polar groups. This theory seems to be incompatible with our observation (18) that in general the dyes of smaller molecular size, which according to Boyd's theory should pull the molecules more closely together, in fact precipitate less completely than those of larger molecular size.

COMPOSITION OF ANTIBODY-ANTIGEN PRECIPITATES AND VALENCE OF ANTIBODY. An essential requirement for agglutination or precipitation according to the framework theory is that both antigen and antibody be multivalent. The

experimental observations which indicate multivalence of antigens and of agglutinins and precipitins have been summarized by Marrack (6), Heidelberger (14), and Pauling (17).

The most straightforward evidence for the necessary multivalence of antigen is given by experiments on the reactions of antibodies with simple substances of known structure. Subsequent to Landsteiner's discovery (10) that simple haptens inhibit the precipitation and agglutination reactions by forming soluble complexes with antibody, it was found by Landsteiner and Van der Scheer (21) that simple substances containing two or more haptenic groups form precipitates with hapten-homologous antibodies. We have shown that of the twenty-seven simple substances containing phenylarsonic acid groups which were tested with antisera made by injecting rabbits with azoprotein made from *p*-arsanilic acid each of those (twenty in number) which contained two or more of the haptenic groups gave the precipitation reaction, whereas none of the monohaptenic substances formed precipitates. These facts support the framework theory strongly.

(The failure to obtain precipitates with some polyhaptenic substances reported by Hooker and Boyd (46) and Boyd (47) may have been due to their failure to work under conditions favorable to precipitation. We have obtained precipitates with some of the same substances, and have observed that substances which give precipitates with strong antisera may fail to do so with weak antisera.)

Experiments which have been reported on the number of haptenic groups per azoprotein molecule necessary for precipitation with hapten-homologous antibody (48, 49) and some of our unpublished results indicate that a few groups are needed, but so far they have not been precise enough to distinguish between 1 and 2 as the minimum.

Direct proof of the bivalence of diphtheria antitoxin is given by the studies of the antitoxin in presence of an excess of toxin with use of the ultracentrifuge, which showed that the complexes ToxAntitox and Tox_2Antitox exist in the solution (50).

The fact that slides can be coated with alternate unimolecular layers of antigen and antibody in specific combination (51, 52) indicates effective bivalence of antibody molecules as well as antigen molecules.

It has long been known that in antigen-antibody precipitates molecules of antibody are present in larger numbers than those of antigen, the antibody-antigen molecular ratio being considerably greater than unity for nearly all systems (6, p. 161; 53). This was shown convincingly by the accurate quantitative investigations of Heidelberger and his collaborators (54). A simple explanation of this fact, which does not follow directly from the framework theory in its original form (6, 14), is given by the theory as modified by Pauling (17), who made the assumption that antibodies in general are at the most bivalent. (This assumption was made because his proposed structural theory of the process of formation of antibodies is such as to make unlikely the occurrence of antibodies of higher valence.) The maximum valence N of antigens toward homologous antibodies is assumed to be determined by the sizes and shapes of the

antigen and antibody molecules, being equal to the number of antibody molecules which, when bonded to the antigen molecule, can be packed around it. If the antigen and antibody molecules were spheres of the same size, this number would be $N = 12$; for smaller antigen molecules it would be smaller, and for larger ones it would be larger.

The predicted antibody-antigen molecular ratio for small antigen molecules would be $N/2$ at the equivalence zone, with the maximum valences of both antibody and antigen effective; the limiting values of the ratio for antigen excess would be 1, and for antibody excess $N - 1$. For very large antigens the expected ratio at the equivalence zone would be less than $N/2$. These predictions are in reasonably good agreement with Heidelberger's observations (54, 17), which correspond to the following values of N: ovalbumin and R-salt-azobiphenylazoovalbumin (molecular weight 40,000–46,000), $N = 6$; serum albumin (m.w. 67,000), $N = 6$ to 8; thyroglobulin (m.w. 700,000), $N = 30$ to 40. Data of other investigators (55, 56, 57, 58, 59, 60, 61) correspond to similar values of N, with assumed bivalence of antigen.

If the valence of the antigen were known, measurement of the antibody-antigen molecular ratio could be interpreted to give the valence of antibody molecules. The only reliable experiments of this sort which have been reported so far are those of Pauling, Pressman, and Ikeda (20) with simple antigens of known structure. They found that the dihaptenic antigens 2-methyl-4,6-di(*p*-azophenylarsonic acid)phenol and 2-methyl-4,6-di(*p*-azobenzene(p-azophenylarsonic acid))phenol gave with antisera homologous to the *p*-azophenylarsonic acid group precipitates with the same molecular ratio throughout the range of relative concentrations from antibody excess to antigen excess. This is what would be expected if both antibody and dihaptenic antigen were bivalent, the predicted molecular ratio under all conditions then being 1, corresponding to the structure —A—B—A—B—A—B—A—B— for the precipitate. The observed molecular ratio (average of 119 analyses) was 0.75. The same independence of molecular ratio on relative concentration was found also for the four trihaptenic and tetrahaptenic substances studied; this was interpreted as resulting from the effective bivalence of these molecules also, as the result of their small size in comparison with the antibody molecules. The average molecular ratios found for the trihaptenic and tetrahaptenic substances, 0.85 and 0.83, respectively, are only slightly less than unity. These results, with assumed effective bivalence of the antigens, indicate for antibody molecules the effective valence 2.3.

Substantiating evidence for the multivalence of precipitating antibodies is provided by the observations which have been interpreted as resulting from the presence in antisera of antibodies with a valence of one. Heidelberger and Kendall (62) demonstrated the presence in rabbit antisera of univalent antibodies, which are able to combine specifically with antigen but are not able, in the absence of multivalent antibodies, to form precipitates. These univalent antibodies also occur in considerable amount in horse antisera; they seem to be produced in large amount by the first injections of ovalbumin into horses, precipitating (multivalent) antibody being formed only on repeated

injection (63, 64). It is probable that univalent antibody confers on horse antitoxins the peculiar properties which they show, in particular the pronounced prezone (region of antitoxin excess in which precipitation does not occur). The change in properties of antisera on heat treatment (65, 66, 67) or treatment with formaldehyde (9) or other denaturing agent is probably due to the conversion of bivalent antibody to univalent antibody by destruction of one of the combining regions.

QUANTITATIVE THEORIES OF THE PRECIPITATION REACTION. Although the quantitative physico-chemical treatment of immunological phenomena was begun early in the present century, by Arrhenius and Madsen (1, 68), it is only during the last decade that significant progress has been made. The reason for the delay is not far to seek—it lies in the fact that the mathematical treatment of numerical data of low accuracy has little significance so long as a sound qualitative understanding of the phenomenon has not been developed. We may mention in illustration Arrhenius' discussion (1, p. 147) of the formula $C = K B^{2/3}$ which he found to express the relation between the amount C of agglutinin bound by bacterial cells and the amount B of free agglutinin; Arrhenius interpreted this equation (whose validity for the complex system we would ascribe to the accidental distribution of the heterogeneous antiserum) as showing that the agglutinin molecules are divided between two solvents, one within and one without the bacterial cells, and that three molecules of the bound agglutinin are formed from two of the free substance.

Recent theories are of two kinds: those based on thermodynamic equilibrium among the reacting substances, and those based on the rates of reactions under non-equilibrium conditions. There has been considerable discussion as to whether or not immunological reactions are reversible—whether, for example, an antigen-antibody precipitate is soluble, and is in equilibrium with free antigen and antibody in solution. We know from general principles, however, that, given time enough, every system reaches equilibrium, and every material is more or less soluble; the questions of interest deal rather with such quantitative points as the length of time required for the system to reach equilibrium, and the magnitude of the solute concentrations in equilibrium with the precipitate.

That the precipitation reaction in some cases reaches equilibrium in the hours or days usually allowed it is shown by various experiments on solution of the precipitate by salt (69), acid (70), alkali (71), and excess antigen, even after ageing for several months (72), including experiments in which there was variation in the method of approaching equilibrium (19).

Experiments on the Danysz phenomenon (73) and other related experiments indicate that a long time—many days—is needed for equilibrium to be approached for reactions involving change in composition of antigen-antibody precipitates.

The first quantitative theory which we shall discuss, that of Heidelberger and Kendall (14), was based on consideration of the rate of antigen-antibody combination under non-equilibrium conditions. The authors assumed that antigen A and antibody B first react completely and rapidly to form the com-

plex AB, which uses up all the A (B being assumed present in excess). There then occur two competing slow reactions:

$$\begin{aligned} B + AB &\rightarrow AB_2 \\ AB + AB &\rightarrow A_2B_2 \end{aligned}$$

The rates of formation of AB_2 (total number of units α) and A_2B_2 (total number of units β) are

$$\frac{d\alpha}{dt} = K[B][AB]$$

and

$$\frac{d\beta}{dt} = K'[AB]^2$$

in accordance with the laws of chemical kinetics. It is assumed arbitrarily that $K = K'$ and that the reactions are not reversible; by integration over the course of the reaction until the solution is exhausted of AB there is obtained to represent the composition of the precipitate, which consists of all the AB_2 and A_2B_2 formed, the equation

$$\frac{y}{a} = 2R - \frac{R^2 a}{b_0} \qquad (1)$$

in which

y = milligrams of antibody precipitated
a = milligrams of antigen added
b_0 = total milligrams of antibody
R = antibody/antigen weight ratio at equivalence point

This equation for the composition of the precipitate, which corresponds to change from $2R$ at large antibody excess to R at the equivalence point, has been shown (15) to be in satisfactory agreement with the excellent experimental data obtained by the authors. In view of the arbitrary and unlikely assumptions originally used for its derivation, it is gratifying that Kendall himself (74) has recently derived the equation in another way, and that we have found (unpublished work) that the equation is obtained as an approximation from general considerations of chemical equilibrium when the assumption is made that the ratio may vary between the limits $2R$ and R and an expansion is made in powers of a/b.

Kendall's derivation is essentially the following. (He considers also some more general cases.)

Let antibody and antigen both be bivalent. For B_0 and A_0 molecules of antibody and antigen, respectively, there are $2B_0$ and $2A_0$ combining groups. Assume, for antibody excess, that all of the $2A_0$ antigen groups are bonded to antibody groups, and that they are distributed at random among the $2B_0$ antibody groups, without regard to whether or not the antibody molecule is already bonded at the other end. Since the chance that an antibody group is

bound is $2A_0/2B_0$, the chance that it is free is $1—A_0/B_0$, and the fraction of antibody molecules free at both ends is $(1—A_0/B_0)^2$. The fraction not free at both ends is $1—(1—A_0/B_0)^2 = 2A_0/B_0—(A_0/B_0)^2$, and the number not free at both ends is this multiplied by the total number, B_0. If it be assumed that all antibody molecules not free at both ends are carried down in the precipitate with the antigen molecules the molecular ratio for the precipitate becomes

$$\frac{B_{pp}}{A_{pp}} = 2 - A_0/B_0 \tag{2}$$

and, introducing the ratio R of molecular weights, the weight ratio is found to be

$$\frac{y}{a} = 2R - R^2\frac{a}{b_0} \tag{3}$$

This is identical with equation 1.

Similar considerations have also been used by Ghosh (75) for the derivation of related equations.

An involved theory of antigen-antibody equilibria based in part on probability considerations has been extensively developed by Hershey (45). The theory, in common with others based on multivalent antigen and antibody, is in qualitative and rough quantitative accord with experiment.

The only theory of the precipitation reaction which, following the program begun by Arrhenius, has been developed by straightforward application of the principles of chemical equilibrium is that of Pauling, Pressman, Campbell and Ikeda (19). This theory applies only to relatively simple systems, namely, those composed of bivalent antigen and bivalent antibody, univalent hapten, certain soluble complexes, and precipitate with invariant composition AB.

In order to show the nature of the treatment, we present here the derivation of the equation for the amount of precipitate formed in absence of hapten, generalized over the earlier treatment by consideration also of the complex AB_2.

Let the molecular species A, B, AB, A_2B, and AB_2 in solution be in equilibrium with each other and with solid AB_{pp}. We represent the concentrations of the five solutes by the symbols

$$\begin{aligned} [A] &= \alpha \\ [B] &= \beta \\ [AB] &= s \\ [A_2B] &= a \\ [AB_2] &= b \end{aligned}$$

The quantities α, β, a, and b are variable, whereas s is constant for a given system with precipitate present; it is the solubility of the precipitate. The equilibrium expressions for the three reactions

$$\begin{aligned} A + B &= AB \\ A + AB &= A_2B \\ B + AB &= AB_2 \end{aligned}$$

are respectively

$$\frac{s}{\alpha\beta} = 4K \tag{4}$$

$$\frac{a}{\alpha s} = K \tag{5}$$

$$\frac{b}{\beta s} = K'' \tag{6}$$

For simplicity we have assumed that each of the bonds in the complex A—B—A has the same strength as the bond in AB; this is probably a good approximation, in view of the fact that the two bonding regions are probably far apart on the large antibody molecules. (The theory can be carried through without this assumption.) The constant K corresponds to equilibrium for one antibody valence and one antigen valence, and the factor 4 is an entropy factor or symmetry factor. We use K'' rather than K for the second bond in AB_2 because steric repulsion between the two antibody molecules attached to the same small antigen would be expected to decrease the stability of this complex.

The expressions for the total amounts of antigen and antibody in the system (per unit volume of solution) are

$$AB_{pp} + s + \alpha + 2a + b = A_{total} \tag{7}$$

and

$$AB_{pp} + s + \beta + a + 2b = B_{total} \tag{8}$$

Subtracting equation 8 from 7 we obtain

$$\alpha - \beta + a - b = A_{total} - B_{total}$$

Eliminating β, a, and b with the use of equations 4, 5, and 6 we obtain

$$\alpha - \frac{s}{4K\alpha} + Ks\alpha - \frac{K''s^2}{4K\alpha} = A_{total} - B_{total}$$

This quadratic equation in α gives on solution

$$\alpha = \frac{1}{2(1+Ks)}\{(A_{total} - B_{total} + [s(1+K''s)(1+Ks)/K + (A_{total} - B_{total})^2]^{\frac{1}{2}} \tag{9}$$

(The positive rather than the negative sign before the radical is seen to be correct by the consideration of limiting cases.) From equation 7 we find on eliminating a and b the expression

$$AB_{pp} = A_{total} - s - (1+Ks)\alpha - \frac{K''s^2}{4K\alpha} \tag{10}$$

Equations 9 and 10 give the solution to our problem; from 9 the value of α is to be found in terms of A_{total} and B_{total} and the parameters of the system s, K, and K'', and this on substitution in 10 gives the amount of precipitate.

It is shown in the original paper how this equation (with $K'' = 0$) accounts for many observed properties of antigen-antibody systems.

Future progress which may be anticipated involves the extension of this straightforward thermodynamic treatment to include the case of variable composition and randomness of structure of the solid phase. This will require the development of satisfactory approximate expressions for the free energy of such a solid phase, by application of the methods of statistical mechanics on the basis of sound structural concepts. This problem is not an easy one; but fortunately promising methods for attacking it have been developed in recent years by able theoretical physicists interested in the problem of the stability of alloys with greater or smaller degree of randomness of atomic arrangement, and we may be confident that great progress will soon be made in the formulation of a satisfactory quantitative theory of the precipitation reaction.

SUMMARY

The forces responsible for combination and attraction of antigen and antibody molecules may be classified as electronic van der Waals attraction, Coulomb attraction, attraction of electric dipoles or multipoles, formation of hydrogen bonds, etc. The specificity of interaction of antigen and antibody molecules arises from their structural complementariness, which permits close contact of the molecules over sufficient area for these weak forces to co-operate in forming a strong antigen-antibody bond.

The weight of evidence indicates that further combination of the initial antigen-antibody complexes to form a precipitate is a specific rather than a nonspecific reaction and is due to a continuation of the primary combination step to form a framework structure of alternate antigen and antibody molecules.

Furthermore it appears that both precipitating antigen and precipitating antibody must be multivalent, at least bivalent.

The more recent quantitative theories of the precipitation reaction are discussed.

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PHYSIOLOGICAL STUDY OF THE VERTICAL STANCE OF MAN

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Many years ago Sherrington (1920) remarked that posture is a theme worth studying, offering much opportunity for observation by those who are willing to undertake it. The literature in the field is rich and varied, spanning several branches of the basic sciences, medicine and surgery. The evolution of the biped stance has received extensive anthropologic study. The mechanics of standing, measurement of postural deformities, their prevention and treatment, have been subjects of comment and investigation for centuries. The neurological mechanisms which control the vertical stance of man and the physiology of the cardiovascular-respiratory responses to the hydrostatic effect of gravity have been exhaustively studied. Much of value to a fundamental understanding of posture may be deduced from a study of case histories which record behavior when controlling mechanisms are pathologically disrupted, or when deformity is sufficient to impair function. It would require a monograph of large proportions to encompass the information available in the literature. Since the subject of posture has yet to be surveyed in this way, the physiological implications of representative papers from the major fields of specialized interest form the basis of this review.

The classical contributions of Sherrington (1915), Magnus (1924a), de Kleijn (1924a) and of Rademaker (1931) on the postural reflexes are well known. Schäfer (1900) and Luciani (1915) have reviewed the early work on animal mechanics. More recent contributions to specialized aspects of biodynamics have been made by Steindler (1935), Morton (1935), Basler (1931), Bernstein (1935), and Phelps and Kiphuth (1932). The relationships of body mechanics to health have been discussed by Goldthwait and his school (1934). A recent symposium on posture was edited by Zirbes (1938) and Hoppe (1928) has contributed a general review.

According to Keith (1923), anatomists do not regard the postural adaptations of the human body as the resultant of a transformation peculiar to man, but rather as the culmination of a series of evolutionary phases which may be traced in the bodies of orthograde primates. After fifty years of interest in this subject he has abandoned his original view that the gibbon is representative of the pioneers of the orthograde stock and that man and the great anthropoids had passed through a hylobatian phase of evolution (1940). He now thinks that while gibbons in their early stages of evolution were developing along brachiating lines, two other modes of orthograde posture were being evolved in the same stock. In one both arms and legs were being used almost equally. In the other the lower limbs were being developed as the chief organs of support and locomotion.

The penalties of poor posture. The change from the quadruped to the biped

posture has imposed difficulties which are interpreted by some as signs of extreme inadequacy of adaptation (Benson, 1938; Hooton, 1936, 1939). Others, while recognizing that the human body is not yet completely adjusted to the erect stance, consider the compensatory powers of Nature admirable and good (Hinchey, 1925; Main, 1937b; Jordan, 1941). It has been widely assumed that anatomical defects in skeletal alignment encroach on body cavities secondarily affecting function (Dickinson and Truslow, 1912; Cyriax, 1938; Baker, 1942), and that good posture is a way to health (Canter, 1932; Forrester-Brown, 1935). At the beginning of the century Keith (1903) postulated that when the abdominal wall is lax and the diaphragmatic supports give way, respiratory balance may be upset. If the inspiratory muscles gain ascendance there is danger of visceral displacement and subsequently, disorganization of function. The major influence on thinking in this field has been exerted by Goldthwait (1932, 1933) who has long believed that the well poised individual is the most efficient because his muscles are well balanced, and because adequate space exists for the best possible functioning of the thoracic, abdominal and pelvic viscera.

Many clinical papers in the current literature on posture indicate that stance defects may result ultimately in a variety of malfunctions including lessened respiratory efficiency, prolapse of the abdominal viscera, impairment of digestion, pressure and derangement of the pelvic organs, dysmenorrhea, hemorrhoids, varicose veins, constipation, cyclic vomiting, foot strain, backache, neuritis and arthritis. Barring orthopedic disabilities, few of the etiologic associations are based on demonstrable fact. Rather, they are accepted as the *a priori* resultants of a sagging chest, low diaphragm, changed position of the heart and general visceroptosis.

Almost from their first enunciation these now firmly entrenched concepts have been questioned (Osgood, 1916). Many observations throw doubt on their validity. It has been pointed out that the variability of visceral position is much greater than implied by textbook descriptions of the normal (Sever, 1913; Baetjer, 1916; Barclay, 1932). On the basis of orthodiagraphic examinations on 900 subjects Mills (1917) considers body habitus a determining factor of major importance to visceral form and position. On the other hand, Coffey (1912) thinks that the development of general visceroptosis is probably impossible in the majority of men regardless of habitus. In about 20 per cent the ascending and descending colon have not completely fused with the parietal peritoneum. Coffey suggests that in this defective fifth of the human race are those in whom floating kidney, mobile cecum and general visceroptosis are to be found. The position of an organ may teach nothing regarding function (Bettemann, 1916), and chronic visceroptosis may exist in the healthy (Abbott, 1934a). Wakefield and Mayo (1937) look upon the digestive function as so fundamental an endowment that it is beyond reason to suspect that the upright position assumed by the human species can in any way hinder, hamper, or decrease its efficiency. Jones (1933) represents the view between the cited extremes. He suggests that the relationship between poor posture and visceral function is "fairly definite" and that by preventing bad posture one accomplishes "a little" toward the pre-

vention of disease. Schwartz, Britten and Thompson (1931) assessed the stance of 2,200 normal men and boys. They came to the conclusion from the unexpected variability of the posture found in this essentially normal and vigorous group, that the importance of posture to health had been exaggerated.

A few direct experimental attempts to relate posture and function have been made. Wheatly and Moore (1927) carried out roentgenographic observations of visceral position and diaphragmatic excursion on a group of young adult women, gave special corrective exercises to those below standard in postural alignment, and note what was interpreted to be an improvement in the factors studied. Klein and Thomas (1931) also reported that specific posture training resulted in demonstrable improvement of their experimental over a control group, the advantage being six to one. Schwartz, Britten and Thompson (1928) had found no appreciable change in posture, for better or worse, when nonspecific exercise was used on a test group of children. Feeling the lack of basic information, Abbott (1934b) applied the Goldthwait postural treatment to patients with asthenic habitus and symptomatic visceroptosis. He found his data difficult to interpret because of uncertainty in the evaluation of postural changes supposedly wrought by the exercises. What is needed in studies of this type is a valid and reliable criterion of good stance, adequate objective measures of anatomic displacement of viscera, and acceptable assays of the functional status of the organ systems theoretically susceptible to influence by gravitational stresses. In few case history studies have there been adequate control observations and therapy has been limited only rarely to correction of posture as the single variable under experimental investigation.

Flagstand and Kollman (1928) used vital capacity as an index of respiratory damage in testing the hypothesis that functional impairment of the thoracic viscera follows lateral distortion of the spine. In mild and moderate abnormalities the vital capacity was within normal limits. In severe alignment disabilities it was reduced to within 53 and 65 per cent of normal. When the curvature was confined to the lower thoracic and lumbar regions of the spine, vital capacity was not affected even though structural abnormalities were severe. Schreiber (1934) made an experimental study on apes to observe the effects of various body positions on the shape of the abdominal cavity. He found a characteristic position of the viscera for each posture studied. Thus topographical pictures may reflect abnormality in posture rather than morphologic anomaly. In 1934 Appleton produced unusual experimental postures in young and adult rabbits. Significant deformations occurred only in the young in which growth was still active.

Laplace and Nicholson (1936) initiated an extensive investigation to determine to what extent the belief that postural defects handicap physiologic function is justified by clinical and experimental evidence. They obtained orthodiagrams of the chest and electrocardiograms, measured vital capacity, respiratory rate, tidal air, pulmonary ventilation, oxygen consumption, blood pressure, heart rate, and circulatory efficiency before and after acute variations in posture. They also made a study of persons who had been taking corrective exercises for

a year. Laplace and Nicholson found that the diaphragm was not always relatively elevated nor the heart more transversely placed by postural improvement. The influence of posture on the excursions of the diaphragm was variable. Vital capacity generally increased. There was no striking difference in oxygen consumption but pulmonary ventilation was greatly augmented. The statistical significance of observed differences was not estimated. They concluded on the basis of equivocal evidence that correct posture appears to have appreciable advantages to the circulation and respiration of most individuals, but that in some an apparent defect in posture may be a compensatory mechanism which it is inadvisable to disturb. Subsequently Nicholson and Laplace (1938) disregard a portion of their experimental data in favor of the *a priori* view that when a good posture is maintained without conscious effort, its metabolic cost is less than that demanded by faulty alignment.

As a whole it has been difficult to displace the idea that attitudinal anomalies must of necessity be associated with functional disturbances. It is inadequately appreciated that organ systems carry on their work within very wide margins of safety, and that many compensatory mechanisms serve automatically to protect vital processes. It is difficult to believe, on the basis of evidence as tenuous as that presented, that the human machine can be so fragile in its construction as to be significantly disrupted functionally by minor skeletal malalignments. Indeed, Rugh (1928) recognized as did Laplace and Nicholson that though postural changes affect appearance, they may be as well signs of compensation aimed at the preservation of essential functions. Möhring (1930) points out the paradoxical fact that a bad posture looks better than a good one if the so-called bad posture is balanced while the good one is not. As early as 1920 Lee and Brown had introduced the term "compensated defects." They looked upon poor posture without symptoms in much the same way as the clinician considers compensated heart disease, neglecting perhaps to take into account the vast difference in the relative importance of these two to reasonably adequate survival. It has been frequently suggested that malalignment may be adequately compensated for in youth, but gives way to symptoms under conditions of strain or as muscle power wanes with increasing age.

The literature following the last war makes repeated reference to the high incidence of disability developing under the rigors of military life and very generally credits faulty body mechanics as a contributory cause (Willard, 1918; Talbot and Brown, 1920; Forrester-Brown, 1926; Milliken, 1928). Hellebrandt et al. (1942) applied more precise modern methods to a re-exploration of the influence of the standard army pack on postural stability and alignment. Industry retains a concern over body mechanics in its relations to fatigue and output (Bedale and Vernon, 1924; Zacharias, 1941) but the contemporary interest of military medicine has wandered away from such problems to an intensive study of the effects of centrifugal acceleration. There is a growing literature in this field. It has recently been reviewed exhaustively by Ham (1943). Positive accelerations of 6 to 8 g are known to occur in the maneuvers of modern combat aviation and human subjects are being exposed to these in controlled observa-

tions in planes and in the centrifuge. When one considers the amazing capacity of the human to resist centrifugal accelerations of very high order, the effects of the gravitational stresses incidental to the simple verticality of the biped sink into insignificance by contrast. That they can never be as malign as implied in a large and uncritical literature on the subject is probably a safe deduction from the type of evidence Ham reviews. Analysis of the "black-out" phenomenon will doubtless add much to our fundamental understanding of the efficacy of the various buffer mechanisms called into play in efforts to avert cerebral ischemia of incapacitating proportions. It is none the less interesting to observe that the application of simple postural tests of cardiovascular efficiency to the selection of prospective pilots was receiving interest as early as 1921 (Scott; Ellis) and that the Schneider Test continues to serve a useful purpose in aviation medicine. The capacity to resist g will doubtless influence the selection of pilots for flight training involving high centrifugal stresses. Factors affecting tolerance to centrifugal acceleration are in need of study (Rook and Dawson, 1938).

Tests and measurements of posture. Efforts to measure posture in objective ways suitable for mass examinations have been made largely by lay observers. In studying the normal, they have been confronted with deviations so slight that elaborate tests have become necessary to assign grades to such variations. In contrast, precision devices have been comparatively little used by the orthopedic specialist in his examination of posture. Since the early observations of Borelli, Braune and Fischer, Vierordt and Leitersdorffer, reviewed by Schäfer and Luciani, few physiologists have interested themselves in the problems of alignment. Those most active in the development of practical methods of assessing the ability of the neuro-muscular and skeletal systems to resist gravity have shown the greatest disregard for the weakness of the evidence already cited which indicts poor posture as a factor inducing malfunction and ill health. Although objective tests are more reliable than subjective ones (Cureton, Wickens and Elder, 1935), justification for making such exceedingly tedious measurements has never been put forward clearly. To date, the tests have been used largely for purposes of cataloguing and education. Few applying them have taken cognizance of the fact that "in biological terms posture is constant, continuous adaptation" and that there is a certain fruitlessness in classifying anomalies, its only satisfaction being the creation of a typology (Campbell, 1935). Few seem to have appreciated the fact that the posture tests in current use are based almost exclusively on an untenably static concept of standing. Recognizing this point, Lee and Brown (1923) concede that the subjective rating of an examining physician who sees the body in use may give a fairer evaluation of stance mechanics than that attainable from a single instantaneous objective observation. However, properly interpreted, posture tests have an obviously useful function.

The literature contains a series of increasingly complex ways of testing and measuring posture. General reviews embracing methods may be found in the papers of Mott (1927), Schwartz (1927) and Babecki (1929). To Clelia Mosher (1915) and Brown (1917) goes the credit for the earliest use of the

schematograph. Fradd (1923) subsequently developed the silhouetteograph, which was later modified to include contours by Hubbard (1935), Wright (1935) and Hallett (1935). Co-ordinates were next included in the photographs (Moore, 1934; Leonard, 1934) to assist in evaluating the verticality of alignment. Biplane stereoscopic photographs were made by Clough and Murlin (1928) and Bernstein (1930) obtained a three dimensional concept of body position with the use of mirrors.

Variations in the depth of the antero-posterior curvatures of the spine have been scrutinized by the use of the lead tape, the conformature (Reynolds and Lovett, 1909), the comparagraph (Korb, 1939) and by the application of light aluminum pointers to project skeletal parts which are invisible in profile view photography (MacEwan and Howe, 1934; MacEwan, Powell and Howe, 1935; Wickens and Kiphuth, 1937). Although these tests yield graphic records, their rating is often based entirely upon inspectional analysis guided by arbitrary criteria influenced unduly by changing esthetic considerations. Even the MacEwan and Howe test, which is one of the most exacting, does no more than replace individual judgment by a group evaluation. That this is inherent in the numerical scoring system proposed has escaped notice.

All posture tests appear to be built around the assumption that the less the jointed body parts deviate from the vertical, the smaller are the rotational stresses demanding equilibration by muscular contraction and the less the energy cost. The speculative nature of this view was recognized by du Bois-Reymond (1909). A vertical line coinciding with the axes of rotation of the joints of the inferior extremities and the lobe of the ear is widely looked upon as the ideal distribution of the parts around the vertical projection of the body's center of gravity. This alignment, which has been called the *normal stellung* after Braune and Fischer, is actually an abstraction never encountered in the normal weight-bearing body of living man. Although it is universally recognized that gravity is the main deforming force acting on the human body for the modification of posture, it is curiously true that few efforts have been made in the field of stance testing to establish an experimentally determined "weight line" as a point of departure for the analysis of the alignment of body parts. This has been done by Cureton and Wickens (1935) by a method identical in principle to that of du Bois-Reymond (1909) and Reynolds and Lovett (1909). Kelso and Hellebrandt (1937), and Hellebrandt and associates have synchronized biplane postural photographs with center of gravity determinations (1938, 1942a, c), and used these combined methods to check the validity of one of the current methods of evaluating stance (Hellebrandt et al., 1942d, e).

The biodynamics of standing. The early work on *Animal Mechanics* of Borelli, the Weber brothers and Braune and Fischer is adequately reviewed by Haycraft (Schäfer, 1900). Recent students in this field include Cotton (1931), Basler (1929, 1932), Okuyama (1932), Kelso and Hellebrandt (1937) and Hellebrandt et al. (1937, 1938, 1942).

"When man became a biped and assumed the upright position, he immediately made an enemy of gravity, and he has been fighting this relentless foe ever

since" (Jones, 1933). In this struggle man demonstrates some of the most striking compensatory automatisms in human physiology. Bedale and Vernon (1924) noted when several positions were taken in duplicate or triplicate on different occasions that there was nothing accidental about the postures assumed. The optimum of any position could be gauged to a nicety and the versions of each mode were hardly distinguishable from each other.

Reynolds and Hooton (1936) were the first to attempt an investigation of the speculative inferences concerning the relation of the pelvis to the mechanics of the erect posture. The degree of pelvic inclination with its associated lumbar lordosis is probably the most important single factor determining the postural attitude of the individual. They observed from a combination of biplane center of gravity determinations and x-ray measurements that asymmetries of the pelvis and unequal leg length bore no relation to lateral displacement of the projection of the center of gravity either in the pelvis or in the base of support.

Mosher (1895; 1919) early called attention to the asymmetry of the natural stance of man. Morton (1935) assumes that the center of gravity plumbs over the geometric center of the supporting base disregarding the toes which he does not consider actively functional in normal standing. There has been interest in the exact location of the vertical projection of the center of gravity on the part of others (du Bois-Reymond, 1900; Basler, 1929; Hellebrandt et al., 1937). Hellebrandt and Fries (1942) direct attention to evidence which tends to invalidate the theoretical conception of Morton. They found that the stance of adult women was overwhelmingly asymmetric, the vertical projection of the center of gravity falling slightly to the left and behind the geometric center of the total supporting base. They propose that this phenomenon of stance eccentricity may be compensatory for a right sided limb preference.

Postural sway is inseparable from the upright stance. This phenomenon has been extensively studied since the pioneer observations of Vierordt (1862). The literature has been well reviewed by Skogland (1942). Gross estimates of the insecurity of the vertical posture were made by Weir Mitchell and Lewis in 1886. The work was continued by Hinsdale (1887) and by Bullard and Brockett (1888). In 1893 Romberg called attention to the diagnostic significance of the inordinate postural sway evoked by simple closure of the eyes and constriction of the supporting base in patients with posterior column disease. Graphic records of postural sway, called cephalograms, are made either by a stylus attached to the head or by strapping a light platform to the subject's back and having him stand under a writing point. Cephalograms have been used for the estimation of postural stability in the studies of Rosenfeld (1915), Eichkern and Skaggs (1928), Skaggs et al. (1932), Skaggs (1937) and Schildbach (1940). In 1922 Miles devised an ataxiameter which permitted better quantitative handling of estimates of postural instability than was permissible with the previous graphic methods. The ataxiameter was subsequently used by Fearing (1924; 1925a, b). In 1940 Liebert combined the graphic and numerical methods, recording the distance covered in the cephalogram. Ricaldoni's (1928) departure in method allowed for a biplane study of the amplitude, fre-

quency, rhythm, and form of the oscillations of the body as a whole while standing. He placed the subject on a platform suspended from cables. The movements of the platform were transmitted to a large air capsule and thence to a recording tambour. Moss (1931) subsequently devised a method similar in principle and this was used by Omwake (1932). Finally, Hellebrandt and Kelso (1942c) obtained oscillograms of postural sway. These compounded the shifts in the center of gravity occurring in the two cardinal vertical orientation planes of the body during natural effortless standing.

There is general agreement that stance is steadied when the eyes are open and focussed on a fixed point, and least stable with the eyes closed. Distraction reduces sway. When the feet are together the stance is unsettled. Turning the toes out to an angle of 45 degrees or separating the feet so as to equalize the coronal and sagittal diameters of support steadies the stance. Sway is much greater in the antero-posterior vertical orientation plane than in the transverse. Height and weight correlate poorly with stability. In the Romberg test the relationships are so low as to lack significance. Thus the body may compensate in other ways for mechanically disadvantageous factors in physical build. Though kaleidoscopic at first sight, when carefully made, postural sway patterns are characteristic for each person and highly reproducible. There is lack of agreement chiefly as to whether stance training reduces postural instability or not, and whether fatigue is reflected as readily as often implied, in an augmentation of sway.

Man possesses a relatively high center of gravity which must be maintained over a small supporting base. Since the weight line falls in front of the ankle joint, shifting gravitational rotatory stresses must be incessantly equilibrated. One might conclude from the inseparable association of postural sway with standing that the struggle against gravity is but poorly met. However, capricious as the behavior of the center of gravity may seem, its oscillations are so accurately balanced that the average relation of the center of mass to the base of support remains remarkably constant, at least in vigorous young adults with good equilibratory and kinesthetic senses (Hellebrandt and Fries, 1942).

The neurological basis of standing. The myotatic reflex is of exceptional importance to the maintenance of the upright stance (Liddell and Sherrington, 1924, 1925). The vertical orientation of the segmented body in respect to the line of gravity is conditioned by a more or less selective distribution of this reflex to the extensor muscle groups. Adjuvant reflexes emanating from widespread receptors, teleoceptive, labyrinthine, proprioceptive and exteroceptive, readily modify the geotonus of the antigravity muscles. Klein and Schilder (1929) aptly present the "postural model of the body" as one compounded from a combination of fluctuating optic influences, tactile impressions and kinesthetic experiences. The commingled sensations which regulate postural tone are only feebly discriminable. They rarely obtrude upon consciousness (Evans, 1926). Fulton and Sherrington (Cowdry, 1930) even place the basic proprioceptive reflex entirely "beyond self-examination by any effort of introspection". Although the brain has only a limited cognitive power over the muscles (Keith,

1933), the acuteness of proprioception is susceptible to cortical influences (Fearing, 1925b). However, posture is essentially an automatism and the mind is unaware of how we do our standing (Sherrington, 1933, 1941).

The orthograde stance of man is a neurological achievement of significance. The multiplicity of the reflexes co-operating in its behalf suggests that the phenomenon must be important to warrant such elaborate protection. Deficiency of one reflex circuit may be associated with a compensatory coaction of those remaining (Hansson, 1932). It is usually conceded that a kinesthetic defect accompanied by insufficiency of more than one of the accessory postural reflexes abolishes normal stance (Wiggers, 1935; Starling, 1936), but the relative importance of the various accessory stance reflexes to the normal adult human has never been definitively determined.

The striking effect of retinal stimulation on the postural tonus of man is readily seen in the Romberg phenomenon. Poorly demonstrable in the normal, the magnitude of the steadying effect of visual impressions when the supporting base is narrowed, is easily appreciated when the proprioceptive reflex circuits are defective as in Fränkel's locomotor ataxia. How the eye controls the tonus of different muscle groups has been beautifully demonstrated in heliotropic insects by Garrey (1918). Unequal photochemical reactions of the two eyes call forth asymmetrical tension, unbalanced postures, and movements along forced paths.

The neck and labyrinthine reflexes have been studied by Magnus (1924; 1926), de Kleijn (1924), de Kleijn and Versteegh (1924, 1927), Beritoff (1915), and McNally (1930, 1933). The attitudinal reflexes originating in proprioceptive receptors of the neck and in the utricle are not conspicuous in adult man, but the tonic neck reflexes are elicitable in the normal human infant during the first twelve weeks of life. Gesell (1938) considers them virtually a universal feature of neonatal infancy. Their persistence beyond the first half year indicates retarded, arrested, or defective development.

Sherrington (1910) believed that centripetal impulses from the touch and pressure receptors of the soles of the feet contribute little or nothing to reflex standing. In the horse, pigeon and cat the afferent conductors could be severed without affecting reflex standing. Harris (1938) has recently described an ipsilateral extensor reflex, mild and long enduring, which may be evoked by stimulation of the medial plantar nerve in the dog. Bard (1941) describes a series of nonvisual placing reactions of importance to quadrupeds in maintaining the center of weight properly within the confines of the supporting base. Little is known about placing and supporting reactions in man. There is reason to suspect that the transformation of the weight-bearing limbs of man into rigid pillars of support by unyielding reflex tonus would be a disadvantageous retention of a primitive reaction incompatible with the maintenance of circulation in the biped.

The predominant afferents responsible for the production and control of postural tonus are those of the proprioceptive group coming from the muscles and joints of the weight-bearing limbs. These are of fundamental importance.

The short circuit myotatic reflexes come into synaptic alliance with the motoneurons of the anterior horn. Their rhythmical bombardment is automatically insured by the incessant stimulation of stretch receptors incidental to the involuntary postural sway with which standing is invariably associated (Hellebrandt, 1938). Thus streams of impulses arise within the muscles themselves. While the stretch reflex is basically a spinal process (Denny-Brown and Liddell, 1927), the afferent flow may impinge upon cord centers significantly modified by descending impulses of supraspinal origin which exert a profound influence on the subtetanic contractions which ultimately develop in the fraction of the antigravity motor units subjected to rotational stresses. The magnitude of the response, its speed and duration, are probably related to the vigor and rate of the stretch, being limited to the units specifically affected. Thus conceived, postural tonus would be automatically adapted to the force which must be equilibrated if the weight bearing limbs are to remain upright (Hellebrandt, Crigler and Kelso, 1939).

The long circuit afferent pathways mediating proprioceptive impulses travel to the cerebellum by way of the dorsal and ventral spino-cerebellar tracts and the posterior columns. Thence these impulses may be shunted rostrally by way of its superior peduncle to the thalamus and the cortex of the cerebrum or downward to synapse with the final common path via the reticulo-spinal, vestibulospinal or rubrospinal pathways. This suggests that the cerebellum, brain stem and cerebrum all contribute to the control of posture. Mesencephalic transection between the anterior and posterior corpora quadrigemini releases the lower motor neuron from the inhibitory influence of suprabulbar centers and throws the antigravity muscles into strong tone. The augmenting impulses emanate from the vestibular nuclei and descend by way of the vestibulo-spinal tracts.

Bieber and Fulton (1938) showed that corticofugal impulses descending from the motor cortex and the premotor cortex are normally concerned in suppressing the neck and labyrinthine reflexes and the righting reflexes of the adult monkey and baboon. The rôle of the cerebellum in the control of postural reflexes is imperfectly understood but Dow (1938) has demonstrated that the anterior lobe has a well defined inhibiting effect on tonus. Bilateral destruction of the red nuclei produces characteristic disturbances in gait, mild increases in extensor and *stutz* tonus, delay and exaggeration of the *schunkel*, *hinkebein* and *stemmbein* reactions. Ingram, Ranson and Barris (1934) conclude that the red nucleus is concerned with the regulation of muscle tone, plays its small part as a co-ordinator, and exercises restraint on certain motor activities in a way perhaps subordinate to influences which originate above the mesencephalon. Mettler et al. (1939) stimulated the corpus striatum of cats and monkeys. Stimulation of the caudate, putamen or claustrum inhibited movements induced by cortical excitation while stimulation of the globus pallidum imparted "plastic tonus" to the cortically induced movements, exerting a holding effect upon them, prolonging their relaxation time.

A variety of efferent conductors impinge on the final common path, tending to increase or decrease the activity of the lower motor neurons. In ascending

the phylogenetic scale, the final common path is brought more and more under the control of cerebral centers. The basal nuclei make only indirect connections with the final common path by way of the red nucleus, the substantia nigra and the thalamus, and efferent impulses from the cerebral motor centers descend either directly or by way of the cerebellum. Thus almost all portions of the central nervous system appear to have some part to play in the control of the postural reflexes. Wilson (1926) doubts whether the skeletal muscles of higher vertebrates ever respond to afferent impulses from one source alone. Indeed, the maintenance of posture must be looked upon as the resultant of a bewildering interplay of reflexes.

The chronological unfolding of the reflexes which allow for the assumption and preservation of the upright posture has been studied recently by Matulay (1938) on a group of 134 children divided into groups according to age. He found the neck and labyrinthine reflexes elicitable by the third month. Between the third and sixth months the hopping and bracing reactions appear and supporting tonus is demonstrable in the lower extremities on passive standing. The tonic reflexes start diminishing during the sixth to the twelfth months and are absent in children between the age of two and one half years and five and one half years in whom righting reflexes, hopping and bracing reactions are fully developed.

Under pathological conditions the primitive reflexes of infancy may reappear (Schaltenbrand, 1928). Weisz (1938) studied the neck reflexes and equilibrium reactions as synergic phenomena. The former fade as the latter increase in strength in the course of normal development. Byers (1938) believes that the primitive postural reflex mechanisms are increasingly buried in higher mammals by the activity of phylogenetically newer neural machinery and reappear only when this is destroyed. Magnus (1926b) has expressed the view that the attitudinal reflexes seen in animals are all present in man but only exceptionally demonstrable in the healthy adult. Neck righting reflexes are present in normal adults (Schilder, 1929). The optical righting reflexes are also active in man. Dusser de Barenne (Murchison, 1934) denies that tonic neck reflexes have been demonstrated in the normal human adult with any degree of certainty. He believes that when they occur, they cannot be identified with the true neurophysiological phenomena of Magnus and de Kleijn, being rather psychogenic motor reactions. Much will eventually be learned from attempts to interpret the postural disturbances seen in clinical medicine in terms of the experimental observations of Sherrington, Rademaker, Magnus and de Kleijn (Brock and Wechsler, 1927; Haynes, 1928; Schaltenbrand, 1929; Wechsler, Bieber and Balser, 1936; Matulay, 1938; Ford and Walsh, 1940; Nielsen and Frieman, 1941).

The energy cost of standing. It has long been a favorite argument of those concerned with the correction of poor posture that so-called good body mechanics bring about a significant saving in energy and hence allay incapacitating fatigue. This concept, like the supposed detrimental effect of poor posture on visceral function, is based almost exclusively on *a priori* evidence. Tepper and Hellebrandt (1938) determined the metabolism of 75 women in recumbency and

passively assumed standing. The average increase on standing was 5.71 cals./sq. m./hr. or 16.25 per cent. Turner et al. (1930) had observed a rise of 5.8 per cent at 62 degrees and 19 per cent at a 90 degree angle from the horizontal. These increases are small in comparison with the metabolic cost of the phasic contractions of exercise, and could hardly be significantly affected by minor alterations in body mechanics (Hellebrandt, Brogdon and Tepper, 1940). Laplace and Nicholson (1936) failed to show real differences in oxygen consumption as a result of improvement in posture.

Sherrington (1915) called attention to the fact that postural contractions may be maintained for long periods of time without obvious fatigue. This astonishing economy of postural contraction gave rise to speculation as to the cause of such indefatigability. Hoefer (1941) concluded from action potential studies that minimum exertion goes into standing under normal conditions. Posture appears to be maintained by the activity of a small fraction of the motor units potentially available. Larger action potentials occur only when weight distribution is changed by shifting or swaying. From this point of view normal standing on both legs is almost effortless.

A rotation of activity among motor units has long been postulated to explain the sustained maintenance of postural contractions without fatigue (Barbour and Stiles, 1912; Forbes, 1922; Fulton, 1926). Bard (1941) argues strongly against this. In part because incomplete tetani can be kept up almost indefinitely without fatigue in mammalian muscle with intact blood supply, Bard concludes "there is no a priori reason for rotation." However, if the stretch afferents are in reality stimulated by postural sway, rotation of motor units probably follows whether there is a priori reason for such rotation or not. A glance at the trajectory of the shifting center of weight during normal effortless standing (Hellebrandt and Fries, 1942) affords good presumptive evidence in support of checkered asynchronism of action on the part of motor units during standing and this may secondarily account in part at least for the indefatigability of postural contraction.

Compensations for the hydrostatic effect of gravity. The gravitational force exerted upon the circulating blood in the erect position is generally recognized as a cardiovascular handicap by virtue of the opposition it offers the venous return. The earliest observations upon this subject were made by physicians describing syncopal attacks. One of these, Piorry (1826), even supplemented his clinical experience with investigative work on dogs. Physiologists long have concerned themselves with the effects of the changes imposed directly upon the cardiovascular system by verticality. They have given much attention to the diverse compensatory mechanisms which exist to offset threatened disparity between the size of the vascular bed and the volume flow in the erect position. A matter so important for survival as the adequacy of the circulation to vital centers located in the head is protected by a number of devices, as are many other indispensable functions. Thus vasoconstriction, acceleration of heart rate, augmentation of respiration, general tonus of muscle, and muscular activity have all been subjects of inquiry in this regard.

There have been many publications reporting a diminution of cardiac output in normal man in the vertical position as compared with the horizontal (Lindhard, 1913; Field and Bock, 1925; Turner, 1927a; Schneider and Crampton, 1934; Donal et al., 1934; Scott, 1936; Neukirch, 1937; Sweeney and Mayerson, 1937; McMichael, 1937; Asmussen et al., 1939b). A few observers have reported values similar for both postures or an augmentation of cardiac output during standing (Grollman, 1928; Schellong and Heinemeier, 1933; Goldbloom et al., 1940; Starr and Rawson, 1941). The magnitude of the reduction has varied from a slight change to a diminution of 26 per cent. It is affected in particular by the length of the standing period, the nature of the change from horizontal to vertical (whether active or passive), the character of the stance (rigid, relaxed, supported or restless), and the ability of the subject to tolerate the upright position under various restricting conditions incidental to the technic of the particular procedure. In their paper, Donal et al. (1934) tabulate the values for cardiac output reported in the literature. The evidence points toward the conclusion that stroke volume is significantly reduced in the vertical stance.

Observations upon postural changes in blood composition and leg volume add indirect supporting evidence to the belief that cardiac output is reduced during standing. They are the consequence of withdrawal of fluid in the dependent parts combined with simple stagnation in a widening vascular bed. In 1928 Thompson, Thompson and Dailey reported an average loss of 11 per cent total plasma volume during prolonged standing. They found this to be protein free. The maximal fluid loss occurred in from 20 to 30 minutes and was returned to the circulation in about the same time upon resumption of recumbency. Waterfield (1931a), using carbon monoxide instead of the dye method for estimating blood volume, obtained comparable values on the quantity of fluid lost, but observed a plasma concentration which suggested that the capillaries became permeable to protein fractions other than the globulin. His plethysmographic measurements of the inferior extremities (1931b) demonstrated volume increases of an order which corresponded well to the blood volume changes, indicating that such loss of plasma from the circulation was due to its leakage into the tissues. Turner (1930) reported a progressive increase in the volume of the legs in the erect position, as did Looke in 1937 and Asmussen and his associates in 1939 (a). Wells et al. (1938) believe the ratio of the final rate of filtration to initial rate is of a magnitude to be expected on the assumption that filtration ceases in some muscles, but continues indefinitely in others and in the skin. The distinction thus drawn between the muscles rests upon their fascial coverings. In those tightly surrounded by connective tissue sheaths, intramuscular pressures rise to 50 cm. of water during quiet standing, which is sufficient to stop filtration. In those muscles more loosely covered, the intramuscular pressure reaches only 20 cm. of water. Thus the latter regions may be conceived of as the filtering areas through which plasma continues to seep into the tissue spaces more or less *ad infinitum*.

The work of Krogh, Landis and Turner (1932) emphasized the relationship between filtration in the capillaries and the colloid osmotic pressure by showing

that when the latter was elevated in standing, the rate of filtration produced by a given venous pressure was uniformly lower. They found a significant leakage of protein in venous stasis when the pressure became high. Youmans et al. (1934) reported a marked increase in the colloid osmotic pressure of serum withdrawn from the foot during passive support at near vertical angles maintained for protracted periods of time. Keys and Butts (1939) also reported a marked increase in colloid osmotic pressure as a result of one-half hour of quiet standing, and confirmed the work of Man and Peters (1933) which showed that the capillaries are impermeable to lipoids. Youmans and his co-workers found that although the leg volume increased, pitting edema did not occur in any strictly normal subjects. However, in spite of the self-limiting effect of filtration, it appears that, as Krogh, Landis and Turner (1932) said, "the erect human being is constantly near to edema."

Further indirect support to the experimental evidence showing a reduction of the total output of the heart in standing is found in the observations of postural changes in blood velocity. Methods used in the study of circulation time in man include the intravenous injection of vital red (Thompson et al., 1928), of histamine phosphate (Bock, Dill and Edwards, 1930), and solutions of calcium, magnesium and sodium salts (Kvale and Allen, 1939; Mayerson et al., 1939). These investigators are in agreement that during verticality a retardation of blood flow occurs. It was more marked when the subjects were passively tilted to the vertical than when they stood, even with as little motion as possible. Subsequent determinations on oxygen utilization of the legs were made by Florkin, Edwards and Dill (1930). They estimated that in the erect posture only one half as much blood flows through the legs in unit time as in the reclining position. Although the superficial veins fill and stretch under the stagnating fluid, it is suggested that the deeper veins are less subject to change in diameter. Kvale and Allen (1939) believe that information on the speed of flow in the arteries can be secured by subtracting the arm-to-tongue time from the arm-to-foot time. The resulting figure, which gives roughly the ventricle-to-foot time, they found increased after 10 minutes in the erect position, indicating slowing of the arterial circulation, apparently as a result of vasoconstriction. Thermostromuhr measurements on dogs (Mayerson, 1942) supplement and extend these on man and provide direct evidence of vasoconstriction with a slowing of volume flow to subcardial regions. The decrease on the arterial side is accompanied by a marked decrease in venous return.

The usual arterial blood pressure findings for normal erect subjects are a systolic pressure equal to or slightly above the recumbent values, and an elevated diastolic pressure. They are accompanied by a heart rate distinctly higher than that in recumbency. In 1904 Erlanger and Hooker observed that the acceleration of the heart and the diminution of the pulse pressure upon the assumption of the erect posture remind one strongly of the effects produced by hemorrhage. These normal postural responses have been widely studied under a variety of experimental procedures. In many cases the subjects have been passively tilted to the vertical to eliminate the complication introduced by the muscular work

of active change in position. The subjects have been suspended in water to counteract the hydrostatic effect of gravity. Observations have been made within 10 seconds after the change in posture and have been continued without interruption through periods prolonged to more than an hour. The subjects have been instructed to stand completely at ease, as quietly as possible, at strict attention, or have been restrained from movement by extrinsic supports. The character of the standing has not always been described. The periods of verticality have been preceded by severe exercise as well as by rest of varying duration in recumbency. They have been interrupted by periods of mild exercise such as walking, either in place or about the laboratory. Environmental temperatures have been either uncontrolled or consciously varied. More papers have dealt with the response of the male than the female.

Within 10 seconds after active change from recumbency to the erect position, the systolic pressure may drop from 5 to 40 mm. Hg as demonstrated by Wald, Guernsey and Scott in 1937. After about 30 seconds, they found that in the majority it regained or passed the recumbent level. The diastolic pressure usually showed a rise with the initial drop in systolic pressure, as did the heart rate. The values were exaggerated if a tilting table were used for the change in position and the systolic pressure then seldom exceeded the recumbent level. When the vertical position was maintained for longer periods, the heart rate rose 14 beats or more per minute and continued to accelerate the longer the period of observation and the more quiet the subject (Barach and Marks, 1913; Sewall, 1919; Mortensen, 1923; Lutterloh, 1937; Turner, 1929; Hamilton et al., 1932; Schellong and Heinemeier, 1933; Hellebrandt and Brogdon, 1938). The systolic pressure was not always held at its original level but frequently fell slightly. The diastolic pressure gradually but consistently rose sufficiently to reduce the pulse pressure to 20 mm. of Hg or less. Gross (1940) reported the oscillometric index as measured in the arteries of the lower extremities to be reduced during standing. Support of the subjects at increasing angles from the vertical has shown the arterial pressure and heart rate values to alter directly with the gravitational stress exerted on the circulation (Turner et al., 1930; Ghrist, 1930; Hamilton et al., 1932; Hellebrandt and Brogdon, 1938). As is to be expected, nullification of the hydrostatic effect by immersion in water holds the values near those characteristic of recumbency (Erlanger and Hooker, 1904; Hellebrandt and Brogdon, 1938; Asmussen et al., 1939c).

Gravity shock—or orthostatic circulatory insufficiency. Not all individuals can tolerate prolonged quiet standing or passive verticality. As Turner emphasized in 1929, circulatory adjustment is effected by each person according to his own pattern, and it is clear that some patterns are far superior to others. Most of the investigators in this field encounter "fainters" among their subjects, even among those classified as strictly normal. They may suffer orthostatic circulatory insufficiency in from 10 to 30 minutes. Their systolic pressure is usually well maintained until just before the point of syncope. The diastolic pressure encroaches seriously upon the pulse pressure, finally falling rapidly as the systolic pressure drops. The heart rate, which may have exceeded the recum-

bent value by 40 beats per minute, suddenly slows. Schellong and Heinemeier (1933) have presented a diagram on the basis of their work with both healthy men and patients, showing the course taken by the blood pressures, stroke volume, minute volume and oxygen consumption in "excellent regulation," "adequate regulation," "the transition period," "pre-collapse," and then infer the data for "collapse" itself. Hick et al. (1940) reported a more marked postural effect in hot environments than did Hamilton et al. (1932) but both groups of investigators rated the circulation less adequate in the erect position than in the horizontal.

Gravity shock has been shown to interrupt standing quickly in post-exercise periods. Mateef (1935; and Petroff 1932) observed it often in sportsmen after sprints at high speed and prevented it by bandaging the legs and thighs. Brogdon and Hellebrandt (1940) found it to be uniformly present in some subjects when they were immobilized in the erect position after a brief vigorous bout of work on a bicycle ergometer. The orthostatic circulatory insufficiency was often rapid in its onset. Heart rates first fell to approximately 115 per minute from exercise values near 200, then began to rise again, approaching the immediate post-exercise level. In recumbency, post-exercise, the fall to near stabilized levels would have been steady. The diastolic pressure elevated, the systolic pressure fell far below the recumbent figure and syncope terminated the experiments.

Weiner (1938) in South Africa combined the factors of exercise and a hot humid environment in studying the ability to stand. He found that most of his adult male Bantu subjects could tolerate an hour of quiet standing after shovelling gravel for an equal period of time. When, in control experiments, the standing was undertaken in a cool room, no cases of collapse occurred.

Compensatory mechanisms must be highly perfected in those able to resist the gravitational stress placed on the circulation in post-exercise standing. Under such conditions there tends to be a great disparity between the volume capacity of the vascular bed and the volume flow of blood. Functional dilatation of the vessels of the inferior extremities increases their volume capacity. The cessation of activity removes the well recognized muscle pump which has assured an augmented venous return during exercise. The hydrostatic effect of gravity tends to further widen the bed and oppose venous return. It is remarkable that the circulation can be maintained under such stringent circumstances.

Mateef and Petroff (1932) and Looke (1937) were interested in the ability to resist gravity collapse as a problem of practical importance to choice of vocations which are carried on predominantly in the upright posture. Massee (1942) has suggested that the ideal occupation for a person with orthostatic hypotension would be that of swimming instructor; standing in water to heart level would alleviate all symptoms.

Postural changes in respiration. The influence of body posture on respiration was recognized by Liljestrand and Wollin (1913). They demonstrated an increased pulmonary ventilation on standing. This they attributed primarily

to an augmentation of respiratory rate. Hamilton et al. (1932) observed that an increase in pulmonary minute volume became evident as their male subjects reached an angle of 50 degrees from the horizontal, which is close to that found by Hellebrandt and Brogdon (1938) to be the critical position at which circulatory embarrassment commonly became evident in young adult women. McMichael (1937) denies the possibility that the increase in pulmonary ventilation is apparent only, secondary to a postural change in pulmonary capacity. The augmentation in pulmonary ventilation cannot be accounted for on the basis of increased metabolism (Turner, 1927; Franseen and Hellebrandt, 1943). Alterations in alveolar carbon dioxide also accompany postural change (Higgins, 1914; Turner, 1927; Böhme, 1937; Main, 1937a). In trying to decide why the alveolar CO_2 falls in the erect posture, Main postulates that the drop is due largely to pulmonary over-ventilation secondary to cephalic ischemia. Hitchcock and Ferguson (1938) propose that the cause of the lowered alveolar CO_2 in the erect posture may be simple dilution resulting from the increased volume of functional residual air (Wilson, 1927), which is then maintained by an impairment of CO_2 transport from the dependent portions of the body due to stagnation of blood in the legs, but Main (1939) and Main and Baker (1941) do not concur in this view.

There are many indications that postural changes in respiration are related to the hydrostatic opposition offered the circulation in standing. Turner (1927) noted that over-breathing occurred especially among subjects who tolerated protracted standing poorly. This observation is supported by McMichael (1937) who found the pulmonary ventilation/100 cc. O_2 to vary approximately inversely as the absolute cardiac output.

Respiratory movements are some times considered as a subsidiary line of defense against orthostatic collapse by virtue of expiratory compression of the abdomen and inspiratory thoracic suction. Hill and Barnard (1897) observed that so long as the vasomotor mechanism is intact, the splanchnic area forms what can be likened to the resistance box to the circulation, and the effect of gravity is of no importance. In the animals studied it was found that the splanchnic vasomotor mechanism was by itself sufficient to compensate for the hydrostatic effect of gravity. However, if the vasomotor tone were abolished, blood collected in the abdominal vessels, the respiratory center was then excited by cerebral ischemia and blood was pumped into the heart by virtue of the aspiratory action of the thorax and the increased intra-abdominal pressure which curbed the outflow through the splanchnic capillaries. Eyster and Hicks (1933) reinvestigated the problem on dogs and concluded that even extreme alterations of respiratory activity fail to modify stroke volume and cardiac minute volume significantly. They suggested that the importance of breathing in reference to venous return has been exaggerated. More recently Boyd and Patras (1941) have reported increases in stroke volume with inspiration which were augmented by the deep and prolonged breathing following vagotomy.

Mettenleiter (1924) called attention to the influence of the pressure changes associated with deep inspiration on the quantity of blood forced from the liver

into the inferior vena cava. Edholm (1942) also believes the gasping respirations of the erect posture act along with contractions of the abdominal muscles to squeeze the liver and force blood up the vena cava. Franseen and Hellebrandt (1943) describe a periodicity in amplitude of breathing during orthostatic circulatory insufficiency so marked as to resemble Cheyne-Stokes respiration. Whether the increase in breathing prolonged the period of tolerance for the vertical posture through aspiratory action could not be determined. The fact that the increases observed were in many cases due to rises in rate with reductions in amplitude renders this unlikely. The augmentation seems rather to reflect a general slowing of the circulation in which the medullary centers suffer hypoxemia unless more effective compensatory mechanisms respond to combat the pull of gravity upon the blood column. Franseen and Hellebrandt (1943) present evidence in support of the material benefit played by postural sway in meeting orthostatic hyperpnea.

In 1937 Main postulated a proprioceptive stimulation of the respiratory center to account for changes in pulmonary ventilation during standing which exceed metabolic needs. Harrison (1939) has also suggested that the increase in ventilation produced by passive exercise or mild activity is due to a reflex arising in the moving parts and affecting the respiratory center. Comroe and Schmidt (1942) likewise conclude from observations on dogs and man that the proprioceptive system definitely contributes to hyperpnea of muscular exercise. Thus one might look upon postural sway as a stimulator of the respiratory center automatically regulating pulmonary ventilation in a manner destined to assist in the combat against the hydrostatic effect of gravity on the circulation. There are those who also recognize the exaggerated respiratory movements of deep breathing as stimuli for a compensatory cutaneous vasoconstriction (Collier et al., 1927; Bolton et al., 1936; Lieb et al., 1936), but it is purely speculative to contemplate whether or not such might contribute in any significant way to the balance of power in the struggle to avert gravity collapse.

Pathological failures in compensation. The imperfections of man's adaptation to the orthograde stance have been abundantly recognized. Although the literature in this field is extensive, the failures in adaptation are only rarely incapacitating to a serious degree. When critically evaluated, they serve convincingly as evidence of the remarkable ability of the human machine to continue its primary functions in the face of handicaps, and to re-emphasize the buffering value of the margins of safety under which all important activities proceed. Failure to isolate posture as the single etiological variable has complicated the interpretation of many reports in the literature. Both the erect posture and prolonged recumbence have been shown to be conducive to the formation of renal calculi (Ward, 1937; Pulvertaft, 1939; Carlson and Ockerblad, 1940; Lich and Mansfield, 1942).

Smith and Eaton (1942) reported a series of cases in which progressive orthostatic tremor of the legs occurred during full weight bearing, while Thomas (1939) observed one in which recurrent transient paralysis of the right arm and leg could be produced by passive tilting to a 75 degree angle. Ephedrine,

benzedrine and paredrine, which controlled the associated postural hypotension, greatly diminished the number and severity of the attacks.

Mosher (1909) developed an instrument for measurement of pelvic obliquity and early stressed the importance of posture to signs and symptoms of abnormal functioning of the reproductive organs in women. She reasoned that intra-abdominal pressure should be directed downward and forward toward the pubic bones and anterior abdominal wall lest the abdominal contents be forced into the superior strait of the pelvis. Lateral obliquity was associated with asymmetry in the position of the uterus. In 1917 she compared the pelvic angle of women with good and poor posture. Faulty habits of standing were associated with a reduction in the obliquity of the pelvis, increase in intrapelvic pressure and retroversion or retroflexion of the uterus. Lateral asymmetries in posture allowed the intestinal loops to slide into the pelvis on the low side and serve as a pry, moving the fundus of the uterus away from the mid-line toward the high hip.

Klotz (1938) stressed the importance of proper weight lifting and weight carrying to the supporting functions of the pelvic floor. X-ray studies demonstrated a more inspiratory position of the diaphragm in incorrect lifting. This suggests that improper lifting may be associated with a Valsalva effort. Proper lifting seems to be done with the glottis open and the diaphragm in the expiratory position. That these points may be well taken is indicated by the observations of Mengert and Murphy (1934) that intra-abdominal pressures may increase from 7.7 to as high as 23.2 cm. Hg by voluntary muscular effort.

In 1927 Miller commenced the use of silhouetteographs in the study of the stance of gynecological patients. He postulated that poor posture produced chronic congestion of the pelvic organs which might then be the cause of leukorrhea and menorrhagia. In 1930 he reported a decrease in the incidence of dysmenorrhea with improvements in body mechanics but subsequently failed to confirm his earlier views (1934). In 1934 Adams concluded that corrective postural exercises resulted in complete or partial relief of painful menstruation in enough cases to warrant their trial where gross pathological lesions could be excluded.

Marx (1928) observed initial and final emptying time of the stomach, lying and standing. The vertical posture inhibited gastric motility. Franseen (1941) observed that gastric acidity was clearly depressed throughout and following periods of fixed standing associated with orthostatic circulatory insufficiency. With free postural sway and improvement of the circulation, the gastric secretory curves were indistinguishable from those of recumbency. She suggested that vasoconstriction in the splanchnic region, while temporarily maintaining an adequate cerebral circulation during rigid standing, might become so extreme as to impair visceral function.

The pathogenesis of orthostatic albuminuria is still poorly understood. Janeway (1917) presents an historical survey of the problem. He found orthostatic albuminuria associated with lumbar lordosis. Brown (1920) and Lee (1923) noted that orthostatic albuminuria was frequently associated with poor

posture in otherwise normal young men. Beer (1937) reported 2 cases which showed massive unilateral albuminuria from the left kidney after maintenance of extreme lordosis for only 10 minutes during cystoscopic examination. He likened this to the lordotic albuminuria of orthostatic origin which is also unilateral and thought to be due to a pinching of the left renal vein between the angle formed by the abdominal aorta and the superior mesenteric artery. Rytand (1937), Russell (1925) and Shannon (1942) present evidence which indicates that orthostatic albuminuria is not always "functional" and that it should not be classified as benign without careful study.

Cordero and Friedman (1928) found the elimination of phenolsulphonphthalein greater in recumbency than during standing. The postural reduction ranged from 10 to 13 per cent and no correlation existed between the amount of water and of dye eliminated. They found the volume output of urine in two subjects with functional albuminuria from 50 to 200 per cent greater in recumbency than standing. Even the sitting posture reduces water diuresis by 36 per cent (Janney et al., 1933). The reduction of urinary excretory rate during standing has been postulated to be due to the number of glomerular capillaries exhibiting active circulation (White et al., 1926), and to the combined effect of increased colloidal osmotic pressure and reduced glomerular filtration rate (Ni and Rehberg, 1931). Smith (1939–40) noted a prompt reduction in renal blood flow in the majority of normal subjects tilted to the 70–80 degree angle, and failure of the filtration fraction to vary inversely with renal plasma flow. McCann (1940) estimated the diminution in renal blood flow of patients with nephroptosis when in the erect posture to range from 20 to 43.5 per cent with relative constancy of glomerular filtration and increase in filtration fraction. This is in contrast to a reduction of less than 10 per cent in normal controls. Asmussen et al. (1939b) noted that diuresis was reduced from approximately 10 cc./min. to 2.8 cc./min. in one subject and from 20 cc./min. to 8 cc./min. in another by prolonged retention at a 45 degree angle during which oxygen consumption was practically unchanged, minute volume decreased from 4.6 to 4 liters, leg volume increased by 480 cc., systolic pressure was well maintained, diastolic pressure rose slightly, heart rate was markedly elevated and serum protein increased 8.5 per cent in spite of high water intake. These investigators also demonstrated that the diuresis could be altered at will by counteracting the hydrostatic effect of gravity on the circulation by immersion in water. They concluded that diuresis was reduced by the upright posture, largely through incomplete reabsorption of water, secondary to a decrease in mesenteric circulation.

Orthostatic hypotension is a recently recognized syndrome related to the vertical posture. It is characterized by a chief complaint of marked weakness on standing, associated with dizziness and syncope. Lowering the head, sitting or lying down are followed promptly by complete relief. The condition becomes progressively worse. Warm weather is associated with an exacerbation of the signs and symptoms. There is usually anhidrosis or hypohidrosis. The vision may be blurred. Diagnosis is predicated on the abrupt decrease in systolic blood pressure to shock levels and failure of the heart to accelerate in response to the

hypotension. Nocturia is frequently present accompanied by decrease in the urinary output in the day time if the patient is ambulatory. The deficiency in excretion of water is associated with no lowering in the excretion of phenolsulphonphthalein. Weis (1935) observed that the total day time urinary output was 1060 cc. with the patient in bed and 380 cc. at night. When up and about the day urine output fell to 430 cc. and night excretion was augmented to 1290 cc. Baker (1938) recorded a day secretion of 450 cc. and night output of 1435 in a typical case. Croll, Duthie and MacWilliam (1935) administered a diuretic dose of water (1000 cc.) and recovered 880 cc. with the patient recumbent against 73 cc. with the patient up and about.

The first authentic case histories of this syndrome were reported in 1925 by Bradbury and Eggleston. A similar syndrome had apparently been described in about 1891 by Laubry as present in a case referred to him by Babinski. The number of recorded cases has now increased to approximately 50, most of them occurring in this country (Ghrist, 1927, 1928; Ashworth, 1929; Riecker and Upjohn, 1930; Sanders, 1931; Chew, Allen and Barker, 1936; Langston, 1936; Browne and Horton, 1939; Farmer, 1941). Several have reported cases of marked postural hypotension which have differed from the syndrome of Bradbury and Eggleston in one major respect, that of compensatory tachycardia (Sanders, 1932; Barker, 1933; Hughes and Yusaf, 1935). It is not improbable that the two conditions are fundamentally different. Gillespie and Barker (1938), for example, observed several children with postural hypotension. The manifestations differed from those already described in a number of significant respects. The orthostatic hypotension was inconstant. It frequently appeared after exercise. There were tachycardia and free sweating. The circulatory insufficiency developed relatively slowly during approximately 15 minutes of standing. Gillespie and Barker credit the differences to age but the signs and symptoms suggest gravity collapse rather than orthostatic hypotension. The differential diagnosis between these two phenomena has yet to be considered in the clinical literature. Gravity collapse is a physiological orthostatic circulatory insufficiency. Orthostatic hypotension is associated with unequivocally abnormal manifestations.

The pathogenesis of orthostatic hypotension is imperfectly understood. The essential defect is in vasomotor regulation and the absent or diminished control of heart rate in response to the sharp change in blood pressure. Alvarez and Roth (1935) made a systematic study of the various sympathetic phenomena in orthstatic hypotension. They pointed out that sympathectomy did not produce orthostatic hypotension unless the lower thoracic anterior roots were sectioned, and suggested that this might indicate that it is the abdominal vessels which fail, not those of the lower extremities (Roth, 1937). Kvale et al. (1939) reported cases of orthostatic hypotension following anterior rhizotomy or bilateral subdiaphragmatic extraperitoneal resection of the splanchnic nerves, celiac ganglia, and the first and second lumbar ganglia in the treatment of essential hypertension. It may be significant that the secondary orthostatic hypotension in these patients was associated with tachycardia.

There have been repeated signs of associated disease of the central nervous system in cases of orthostatic hypotension (Ganshorn and Horton, 1934; Ellis and Haynes, 1936; Baker, 1938). Laufer (1942) observed the syndrome in a subject with influenzal encephalitis with central nervous system residues. Spingarn and Hitzig (1942) described orthostatic circulatory insufficiency in tabes dorsalis and Addison's disease, and MacLean and Horton (1937) report a case associated with myasthenia gravis. Stead and Ebert (1941) concluded that orthostatic hypotension is a selective disease of the sympathetic nervous system, probably of central origin, sparing some parts. One of the 3 original cases of Bradbury and Eggleston came to postmortem examination in 1927 but the brain and cord were not studied. There was no anatomical evidence of sympathetic involvement, suprarenal disease or status thymicolymphaticus.

Kvale et al. (1939) made circulation time determinations in orthostatic hypotension and reported no reaction within 2 minutes when standing, indicating marked stagnation of blood in the veins. Hallock and Evans (1941) reported a case of hypotension with tachycardia in which they observed an exaggeration of the normal filtration process during standing. Within 10 minutes after being tilted to the vertical posture there was an absolute decrease of 567 cc. or 11 per cent in plasma volume and 650 cc. or 7.5 per cent in whole blood. The normal plasma reduction in 10 minutes was noted as 80 cc. There was hemoconcentration. There were rises of 5 per cent in hemoglobin concentration, 6.5 per cent in hematocrit, and 14 per cent in plasma protein. Stead and Ebert (1941) were unable to confirm the observation of abnormal filtration rate. They found that pooling of blood in the abdomen alone did not produce a striking fall in blood pressure, nor was the pooling in the extremities sufficient to account for the hypotension. When the subject stood in water, there was no pooling of abnormal amounts of fluid in the lower extremities. Stead and Ebert concluded that the essential defect in the patient with orthostatic hypotension is an abnormal response to the pooling of normal quantities of blood during standing, the definitive feature being an absence of vasoconstriction in other parts of the body when the vascular bed in one part is dilated.

Corcoran, Browning and Page (1942) believe that failure of the renal pressor system may participate in some measure in the genesis of orthostatic hypotension. They tilted a patient with postural hypotension associated with tachycardia to 60 degrees and observed in addition to the decrease in systolic pressure progressing to syncope, a diminution of renal blood flow. Angiotonin was then administered, greatly increasing both the blood pressure and renal blood flow, in contradistinction to the renal ischemia manifest in normal subjects under like conditions. Angiotonin is the effector agent of the renal pressor system. Under usual circumstances it excites renal arteriolar vasoconstriction sufficiently to reduce renal blood flow. Corcoran et al. interpret their findings to mean that in the type of orthostatic hypotension under observation, the normal autonomy of the renal circulation is lost.

The subject of Corcoran, Browning and Page's observations slept for two months in the head-up bed of MacLean and Allen (1940). This brought about

a progressive delay in the onset of postural syncope. Eventually, standing while doing light work was tolerated without symptoms. When the patient was now tilted to 60 degrees, syncope no longer occurred and there was an increase in renal blood flow over that originally observed. The administration of angiotonin was followed by a pressor response with renal vasoconstrictor effects indicative of return to normal.

Sewall (1916, 1919) early tried the use of an abdominal support in the treatment of orthostatic vertigo. As a whole, abdominal binders, tourniquets and bandages on the thighs have been without significant beneficial effect in the treatment of orthostatic hypotension (Barker and Coleman, 1931; Ganshorn and Horton, 1934; Alvarez and Roth, 1935). Allen and Magee (1934) reported that thigh cuffs decreased the hypotension when this could not be obviated by the use of an abdominal binder. Hughes and Yusaf (1935) report a case history in which relief was obtained by walking. Allen and Magee (1934) describe a patient who discovered he could temporarily avert syncope by voluntarily contracting the muscles of his legs. MacWilliam (Croll, Duthie and MacWilliam, 1935) believes that both pulse rate and blood pressure adjustments in the standing posture are influenced by afferent impulses from the lower extremities "apparently originating in some part of the vascular circuit in these limbs." When a patient with orthostatic hypotension was made to execute a series of slight, slow movements, shifting the feet and progressing by minute steps, there was a notable alleviation of the hypotension. MacWilliam concluded that it was not simply the erect posture *per se* which explained orthostatic hypotension, but posture plus immobility of the lower extremities. Laplace (1942) described an atypical case in which hypotension could be abolished by increasing the carbon dioxide content of the inspired air or arrest of the circulation to the active muscles. The rationale of the latter may be explained in terms of the blood pressure elevating reflex of Alam and Smirk (1937) arising from voluntary muscles.

Jeffers et al. (1941) classified the types of postural hypotension and discussed their treatment. Most success has been obtained through the use of the sympathomimetic amines on the assumption that orthostatic hypotension is associated with disturbance of the adrenergic portion of the autonomic nervous system. The following drugs have been shown capable of ameliorating the symptoms: ephedrine sulphate (Weis, 1935), benzedrine (Korns and Randall, 1937; Davis and Shumway-Davis, 1937; Brewster, 1940), and neosynephrine hydrochloride (Capaccio and Donald, 1938).

In 1940 MacLean and Allen described two cases of postural hypotension associated with high heart rate to which they gave the term orthostatic tachycardia. One occurred secondary to sympathectomy for essential hypertension and the other demonstrated a less abrupt fall in blood pressure than usual in individuals with orthostatic hypotension. That these patients probably exhibited some of the cardinal characteristics of physiological orthostatic circulatory insufficiency is indicated by MacLean and Allen's conclusion that the defect was not in arteriolar vasoconstriction but rather a failure in maintenance

of an adequate venous return. This defect in the "potential" venous return was diagnosed by the Flack test (Flack, 1923) which is essentially a Valsalva effort, the obstruction to the inflow of blood from the venous cistern of Keith by a high intrathoracic pressure being sufficient to induce syncope during the test when combined with an already defective venous return. Because the symptoms were worse upon arising in the morning than at the end of the day, MacLean and Allen postulated that something incidental to recumbency of the night was responsible for the recurrent loss of adaptation to the hydrostatic effect of gravity on the circulation. With this in mind, they placed the patient in a head-up bed. The results were startlingly good. In 3 days one could stand for a half hour without syncope and in 4 days the other experienced no distress standing for one hour. Both showed return of postural syncope after one night in a flat bed. The mechanism of this unusual effect is yet to be determined.

Fine and Miller (1940) describe a bizarre case of paroxysmal auricular tachycardia which occurred invariably in sitting or standing unless under quinidine or digitalis control. It was explained by the assumption that a potential ectopic focus becomes activated in the erect posture as a result of diminished vagal activity induced by the lessened blood flow to the head which influences the center both directly and by way of the carotid sinus. In spite of the tachycardia, postural variations in blood pressure fell within normal limits. Weiss has made an extensive study of the carotid sinus reflex in health and disease. Weiss and Baker (1933) observed that pressure on the carotid sinus regularly brought on fainting more quickly when the patient was standing than when recumbent. The time interval between stimulation of the sinus and the onset of fainting was measured repeatedly in various positions of the body. It required 30 sec. to bring on the attack with the head down and the feet elevated to an angle of 30 degrees; 10 sec. in the horizontal and 8.5 sec. while standing. The symptoms incidental to a hypersensitive state of the carotid sinus reflex almost always occur when the patient is in the upright position and are relieved by his lying down when premonitory symptoms appear (Weiss et al., 1936).

Åkesson (1936) made an extensive study of changes wrought in the electrocardiogram by the assumption of the vertical posture by individuals without evidence of heart disease or intoxication. He selected 40 cases each of the most hypersthenic and most asthenic in habitus from a group of 200 normal defense workers. There was inversion of the T_2 or T_3 wave or reduction in its amplitude in 31 per cent of the cases. The more marked the grade of asthenia the greater the change in amplitude of the T wave. In contrast there was inversion of T_2 or T_3 in 69 per cent of 45 patients with a clinical diagnosis of "orthostatic anemia." Åkesson concluded that the change in position of the heart in standing and hence in contact with adjacent conducting media, could not be taken as sufficient to explain the findings. Perschmenn (1939) concurs in this. T wave changes developed slowly in his subjects who were maintained at a passively supported posture for prolonged periods of time. There was no evidence of hypoxemia of the heart muscle and no relation between the blood pressure in the

vertical posture and the electrocardiographic change. There is general agreement that the typical electrocardiographic change due to shift in posture from the horizontal to the vertical is a flattening or inversion of a formerly positive T wave in leads 2 or 3 and this occurs especially in the young of asthenic habitus in whom there is vasomotor lability (Schlomka and Reindell, 1936; Erkelens, 1937; Hinrichs, 1937; Janzen, 1938; Sigler, 1938; Ylvisaker, 1940; Scherf and Weissberg, 1941; White et al., 1941). White et al. believe the position of the heart is the most important factor in the production of the T wave inversion. Mayerson and Davis (1942) suggest that the changes occurring beyond the immediate ones traceable to altered heart position are due primarily to increased sympathetic activity during the period of standing.

Adaptations for the prevention of orthostatic circulatory insufficiency. The problem of compensatory mechanisms for gravity collapse was first studied systematically in the laboratory by Leonard Hill in 1895. In his classical experiments, rabbits, cats and dogs suffered marked impairment of the cerebral circulation when held in the vertical position. The effect was exaggerated when splanchnic vasoconstriction was prevented. Monkeys were better able to tolerate passive verticality than rabbits, cats or dogs, but also lost the power to do so with impairment of vasomotor function. Vasoconstriction is considered by many the chief form of compensation which normally occurs in the erect posture, operating to prevent pooling of blood in the viscera and dependent limbs. In man the diastolic pressure has been shown to rise during standing. This is a sign of increasing vasomotor tone. It is compensatory in nature until it encroaches too far upon pulse pressure. The reflexes originating in the vascular presso-receptors are responsible for this control. For some people it seems that the carotid sinus reflex is of less importance in the maintenance of blood pressure in the erect position that it is for others (Berry, Horton and MacLean, 1940).

There have been efforts to evaluate peripheral vasoconstriction as a factor of compensation in the erect and tilted posture, but the results are at variance. Roth et al. (1938) found an increase in skin temperature of the toes in the erect position, in contrast to the report of Youmans et al. (1935) that there occurred a prompt and significant fall in temperature. Roth and her associates attributed the discrepancy to the fact that the subjects of the earlier experiments were not in a basal condition. However, Nielsen et al. (1939) and Mayerson and Toth (1939) also report evidence of peripheral vasoconstriction with passive change of posture, confirming the conclusions of Youmans. Both made observations at high environmental temperatures. Nielsen et al. consider that the disturbance of the heat regulatory function due to vasoconstriction in the tilted position is to a certain degree compensated by an increase in evaporation. Mayerson and Toth also present evidence that reflex vasodilatation gives way to postural change. With heating of the extremity, the postural effect was at first overcome until complete vasodilatation was achieved, at which point the postural change again became evident. They found no significant differences between "fainters" and "non-fainters" in this respect, indicating that syncope in some cases may be due to failure of compensatory mechanisms other than vasoconstriction.

Attention also has recently been drawn to the venomotor system by Weiss, Wilkins and Haynes (1937) and Wilkins, Haynes and Weiss (1937), in studies concerned with peripheral circulatory failure. The administration of sodium nitrite induced an orthostatic collapse in healthy young adults who had experienced no subjective symptoms when the drug was given in the horizontal position. The work demonstrates that elements of the vascular bed peripheral to the arterioles in the extremities, as well as the abdominal vessels may act as regions for the pooling of blood in the upright posture.

Cardiac acceleration is a universal finding in the erect position. Hill (1895) considered this as a subsidiary compensation. The mechanism of its production as well as that of vasoconstriction has been clarified in part with the description of vascular receptors sensitive to hypotension. Diminutions in carotid pressure, adequate to influence the cardiac reflex centers, have been shown by intra-arterial determinations to occur in human subjects tipped to the vertical (Loman et al., 1936). Amussen, Christensen and Nielsen (1939) relate the rise in heart rate to volume changes of the extremities and the resultant decreased venous return of the vertical position. They considered the change of heart rate as essential in the adaptive mechanisms attempting to hold the blood pressure at levels compatible with an adequate circulation. Two summaries of their work may be found in the English literature (Krogh, 1939; Asmussen, Christensen and Nielsen, 1940). The height to which the heart rate rises in standing may be considered in inverse relation to the adequacy of other compensatory mechanisms and as such is incorporated into postural tests of circulatory fitness proposed by Crampton (1913), Schneider (1920) and Turner (1927b; 1929). In 1897 Hill, Barnard and Soltau observed that when a man is in vigorous health the effect of gravity is compensated for by the vasomotor mechanism and not by the heart. When fatigued or neurasthenic, compensation occurs by virtue of an exaggerated increase in heart rate.

Muscular contraction is clearly an influence of importance in the regulation of the circulation during exercise. The pumping effect of rhythmic compression and release of the capillaries and valved veins is commonly recognized as decisive in assuring the venous return requisite for an augmented minute volume during intensive physical exertion. Hill, Barnard and Soltau (1897) noted in observations on healthy men, that venous tension might be grossly revealed as rising while a limb was held dependent and immobile, but that the condition of the limb changed during activity of the appendage. Youmans et al. (1935) showed that active muscular movements are accompanied by less concentration and less rise in colloid osmotic pressure of the blood of the active extremity than the one kept motionless during the experiment. Stead (1940) came to the conclusion that everyone will eventually faint if suspended long enough without support. He remarks that in death by crucifixion the victims were frequently tied to the cross, and the onset of collapse and syncope was hastened by the giving of wine, which caused further relaxation of the vascular system.

Aside from eliminating muscular activity through the use of tilting boards for passive support in verticality, little specific attention has been paid experimentally to the slight but continual movement which is invariably present

when the nornal biped stands and which may be a decisive factor in the maintenance of an adequate minute volume in the erect posture. Its efficacy should differ only in degree from the maximal squeezing effect operative in sport and that minimal which is compatible with normality as represented by skeletal muscle tonus.

Yandell Henderson (1931) suggested, in an amplification of his veno-pressor concept, that the general tone of all the skeletal muscles as well as the non-striated is a factor of great importance in venous return. In 1936, with Oughterson, Greenberg and Searle, he described a method for measuring intramuscular pressure and produced evidence of its influence on the circulation. A method similar in principle was applied by Mayerson and Burch (1940) to the study of peripheral pressure changes in men supported at a 75 degree angle. A considerable hydrostatic pressure is added to that in the veins of the inferior extremities in such a position. McIntire and Turner (1935) had found that the pressure in the veins of the foot was somewhat greater than the hydrostatic factor in healthy subjects who experienced no dizziness in a tilt to 75 degrees. In one subject who exhibited poor circulatory adjustment the pressure in the veins was insufficient to overcome the hydrostatic factor. Mayerson and Burch report an immediate and simultaneous rise in venous, subcutaneous and intramuscular pressure upon change in posture. Subjects who did not develop syncope showed a secondary and usually more marked increase in intramuscular pressure during the upright period, an increase which was absent in those demonstrating circulatory embarrassment. Increasing tonus by muscular contraction eliminated all signs of syncope. The experiments of Mateef and Petroff (1934) upon subjects with pathological loss of tone showed them to be particularly susceptible to gravity shock unless protection was afforded by bandaging from sole to pelvis. Their subjects with pathologically high tone tolerated as much as 55 minutes of standing with comfort and only moderate increases in heart rate. Such observations emphasize the importance of muscle tone in preventing dilatation of the capillaries and advancing blood toward the heart.

Hooker in 1911 pointed out that muscular action capable of emptying veins may be due to involuntary contractions. Many investigators have remarked since upon its possible assistance to the circulation. Mateef and Petroff (1932) noted periods of lability in the cardiovascular reactions to protracted standing which they thought might be associated with insignificant body movements. MacWilliam (1933) has postulated that slight, repeated movements of the weight-bearing limbs such as very slow progression with minute steps, influence pulse rate adjustments to standing through afferent impulses, but seems to ignore the mechanical effect of such movements in aiding venous return.

Looke (1937) remarked that in occupations carried on in the standing position, the slight activity of the leg muscles suffices to restrict too serious an increase in leg volume. Hellebrandt, Crigler and Kelso (1939) were able to demonstrate transient changes in intramuscular pressure during normal comfortable standing. They concluded that the rhythmic variation of intramuscular pressure was probably due to a shift in the incidence of tension between different

antigravity groups during comfortable relaxed standing. Hellebrandt and Brogdon (1938) have compared the cardiovascular response during standing with normal physiological sway permitted or restricted by extrinsic supports. Only when postural sway was unrestrained, could an hour of standing be tolerated without marked acceleration of heart rate, diminution of pulse pressure and eventual syncope. Postural sway was also shown to be a factor of material benefit in combating the orthostatic hyperpnea of normal subjects (Franseen and Hellebrandt, 1943) and to serve as an adequate preventive of post-exercise gravity shock (Brogdon and Hellebrandt, 1940).

The architectural design of the human is not conducive to stability. A segmented structure, its center of gravity is placed high above a relatively small supporting base. Postural sway is inseparable from the vertical stance of man. This postural sway, autonomously controlled by the short circuit myotatic reflexes of the antigravity muscles, may be conceived of as playing a decisive rôle in the maintenance of an adequate venous return in a position as disadvantageous to the circulation as verticality. The small bulk of the antigravity muscles of the stable quadrupeds used in animal experiments can hardly be expected to play a very important part in the adjustment to vertical postures foreign to the species. Other compensatory mechanisms must be for them relatively more important. In man, however, the triceps surae, quadriceps femoris and the glutei represent an impressive share of the total muscle weight. Even partial activity of such a mass may well supply motive power against gravity to a significant quantity of blood. There is probably sufficient evidence to suggest that postural sway, acting as an accessory pump, is a more important compensatory mechanism for the prevention of orthostatic circulatory collapse than has been generally recognized. Indeed, without it, adequate venous return is difficult to maintain even in the healthy when the vertical stance must be sustained for protracted periods of time.

SUMMARY

The evolution of the biped stance has been marked by a narrowing of the base of support and a progressive elevation of the center of gravity of the body as a whole. Both mitigate against stability. The vertical posture also imposes an hydrostatic handicap which encroaches enough on the adequacy of the circulation to make man in the upright stance vulnerable to peripheral circulatory collapse. The numerous difficulties seemingly attributable to the change from quadruped to biped standing are interpreted by some as signs of extreme inadequacy of adaptation. However, compensatory mechanisms automatically cancel the apparent mechanical disadvantages of the change so that gravitational stresses are counteracted easily in the majority of normal men.

Posture has been studied by a variety of graphic and numerical methods which penalize angular deviations from strict verticality. The common attitudinal anomalies are widely conceded to encroach sufficiently on body cavities to impair visceral functioning. They are frequently associated with an augmentation of gravitational rotatory stresses which are assumed to necessitate ex-

haustive muscular contraction for adequate equilibration. Neither view is supported by convincing experimental evidence.

The steadiness of standing is affected little by mechanical factors in build. Although the center of gravity of the body as a whole shifts incessantly during relaxed and effortless standing, the patterns formed by a trajectory of the shifting center of weight and the mean position of the vertical projection of this theoretical point are relatively constant. The phenomenon of standing is elaborately protected by a multiplicity of co-operating reflexes. Primitive automatisms present in the quadruped may also be observed in neonatal infancy, after which they are submerged by phylogenetically newer controls that are capable of being lifted by disease.

Gravity is the major deforming force affecting man's stance, and postural sway a constantly varying stimulus to the stretch afferents. In this way tonus is automatically adapted to the collapsing forces which must be equilibrated by the antigravity extensor muscles. The final common path through which peripheral control of standing is mediated is also subject to an inhibitory modulation by way of motor and premotor corticofugal and cerebellar paths, to excitatory regulation by the vestibular nuclei, and to less well defined influences emanating bilaterally from the red nucleus and the corpus striatum.

Standing is cheap in terms of metabolic cost and it has yet to be demonstrated unequivocally that improvement in so-called body mechanics is associated with a significant decrease in energy exchange. The remarkable indefatigability of relaxed standing has not been fully explained. The sensation of fatigue undeniably associated with prolonged standing is probably the resultant of more or less acute hypoxemia of higher centers which exercise a controlling influence over muscle tonus and cardiovascular respiratory mechanisms. The hydrostatic oppostion to venous return, loss of fluid into the tissue spaces of dependent parts, reduced velocity of blood flow and diminution in cardiac output act as multiple stimuli to a variety of compensatory mechanisms the objective of which is to offset the disparity between the size of the vascular bed during standing and the volume flow. The relative importance of vasoconstriction, cardiac acceleration, augmentation of respiration, skeletal muscle tone, and insensible contraction, to the maintenance of an adequate circulation during standing in man, has not been determined. Self-regulating involuntary postural sway may occupy a position in the primary line of defense by virtue of mechanical effects and reflex influences upon the vital centers located in the medulla. Most of the compensatory mechanisms have been studied under conditions of graded gravitational stress, immediately after postural change, and during standing prolonged to the point of orthostatic circulatory collapse. Gravity shock is aggravated by previous exercise and high environmental temperatures, both of which augment the disparity between the volume capacity of the vascular bed and the volume blood flow.

Orthostatic hypotension, tachycardia, albuminuria, oliguria, flattening or inversion of the electrocardiographic T wave, depression of the motor and secretory functions of the stomach, and dysmenorrhea have all been attributed

to the vertical stance. Failures in adaptation are occasionally so acute as to give rise to syndromes of pathological significance, but it has yet to be demonstrated that when these occur posture plays a decisive etiologic rôle.

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NON-CALORIC FUNCTIONS OF DIETARY FATS[1]

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Although it is well known that dietary fats can be largely replaced by carbohydrates without any immediate and striking ill effects upon experimental animals, a sudden decrease in the fat content of human diets brings complaints from workers that they are unable to do as much work and their appetites are not satisfied. Starling (177) stated in 1918 that the people lost weight rather than eat more of the high carbohydrate diet made necessary by the war-time shortage of fats. Now, a new generation is faced with the same problems of fat-rationing and it seems desirable to reconsider the value of fat in the diet in the light of recent investigations The excellent review by Anderson and Williams (3) in this journal five years ago makes it unnecessary to go over the whole field again. The fundamental ideas about fat metabolism are essentially unchanged and it is the object of this paper to bring the subject up to date, citing the new data which confirm or deny current theories. The average American consumes about one hundred pounds of fat annually, deriving therefrom approximately one-third of his total calories. This is a result of natural selection of foods according to appetite and may be considered normal. Our most concentrated energy source, fats, can supply the needs for the heaviest work without undue bulk.

Experience shows that the growth and reproductive performance of animals is modified by the consumption of dietary fats, which differ among themselves in their effects. Since all natural fats are glycerides of fatty acids any observed differences must be attributed to 1, the acids themselves; 2, the glycerides, or 3, substances which are associated with the glycerides as impurities or as a part of the lipid molecule itself. Those properties of the fatty acids, glycerides and known impurities which may conceivably account for the demonstrated effects of fats will now be considered.

Digestibility. It goes without saying that a food can only be as efficient as it is digestible and in this respect the average fat ranks high. Many experiments with man and laboratory animals have shown digestibility coefficients of over 97 per cent. It should be noted that figures in the literature are not always obtained by the same method of calculation. In some cases the values are not corrected for endogenous fat (the fecal fat of controls on fat-free diet).

Some factors which affect digestibility are 1, species of animal; 2, health of the animal; 3, amount of fat in the diet, and 4, melting point and composition of the fat. Method of feeding, presence of roughage and other variables, excepting high calcium, seem to have little effect on the results. The present discussion is limited to digestibility in its usual sense. Factors affecting rates of absorption and theories of transport across tissues have been recently reviewed by Frazer (68) and by the authors (26).

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McCay and Paul (135) have shown that the guinea pig is much less able than the rat, rabbit or sheep to utilize hard fats. The rat compares favorably with the human being in this respect and by data obtained on the rat the usefulness of a fat to man may be judged.

The health of the subject is essential to utilization of fat or other foods. Obstruction of the bile duct has the most striking effects on fat absorption but other factors affect the retention of fat to a marked degree. Many people are sensitive to fats, which must, therefore, be limited in the diet. Premature infants and twins have difficulty in absorbing fats, especially the more saturated ones (185). Langworthy (108) pointed out that a number of fats, particularly beef suet and cocoa butter, exerted laxative effects on the subjects (human) if fed at high levels. Gullickson, Fontaine and Fitch (77) found that young calves suffer severe scours when certain liquid oils are homogenized into a skim milk diet. These laxative effects were not noticeable if the amount of fat was kept small. Langworthy's subjects were able to consume as much as 116 to 130 grams per day with no laxative effects. The rat tolerates fats extremely well as shown by the experiments of Hoagland and Snider (91) in which digestibilities were about the same in diets containing 5 and 55 per cent of fat.

Of all the factors affecting digestibility hardness or melting point has received the most attention. There is a point above which fats are so poorly utilized that they are not only lost as food but also exert deleterious effects upon the animal. Instead of the 97 per cent digestibility of liquid oils and soft fats the utilization of harder fats, either natural or synthetic, is likely to fall gradually as melting points rise above body temperature, until at 50°C. only 90 per cent retention by man may be expected. With strictly comparable triglycerides the effect of melting point can be strikingly demonstrated with rats. For example, Evans and Lepkovsky (64) found the following digestibilities in per cent for palmitin (m.p. 58–60°C.), myristin (m.p. 53–54°C.), laurin (m.p. 43°C.), caprin (m.p. 25–26°C.), and caprylin (m.p. 7–8°C.), respectively: 73.4, 91.0, 96, 96.5 and 97.5. It will be seen that an important loss in digestibility has occurred at a melting point of 53°C. In an attempt to correlate digestibility with the melting points of mixed fats Hoagland and Snider (92) studied a group of lards, hydrogenated vegetable shortenings and shortenings made of mixed animal and vegetable fats. In the range from 39°C. to 56°C. no correlation was found between the percentage utilization and the melting point, or amount of total saturated acids in the fats. There were, however, large differences in digestibilities as shown by the following data: average for lards 94 per cent, average for hydrogenated vegetable shortenings 87.5 per cent, and average for vegetable and animal shortenings 85.6 per cent. These are significant differences for which an explanation may be found in some of the following facts. The excreted fatty acids are of very low iodine number, showing a selective excretion of the saturated acids which must, therefore, to a considerable extent affect digestibility. Holt and co-workers (96) were able to predict the digestibility of fats by infants by assuming the following percentage retention of the fatty acids: Unsaturated, 98; short chain saturated, 90; palmitic, 88; stearic, 60; and saturated acids longer than stearic, 40. Barbour (8)

found the arachidic acid (m.p. 76°C.) of peanut oil almost wholly excreted in the feces along with appreciable amounts of stearic and palmitic acids. Hence digestibility depends more upon the amounts of longer chain saturated acids than upon the total saturated acids. Hydrogenation may have produced stearic acid without increasing the total saturated acids beyond the value for the lards, which are much richer in the more readily absorbed palmitic acid.

One other point is of interest here. The same acid in compounds of different melting points will be absorbed to different degrees as shown by the ready utilization by rats of ethylstearate (m.p. 30–31°C.) as compared with stearin (m.p. 71–72°C.) (64). However, there are numerous reports of poorer utilization by other animals of ethyl esters as compared with the glycerides (96). The melting point of a mixed fat is not a good criterion of its composition. A difference of several degrees in melting points of glycerides arises from a shift of a fatty acid from the α to the β-position (41). The decrease in *in vitro* hydrolysis by pancreatic lipase of hydrogenated fats of increasing melting points correlates well with the observed fall in digestibility (186).

It is evident from the cited data that the hardness of fats and their content of long chain saturated acids exert a material effect upon their food value. Melting point, as determined by the official methods, is not an exact indicator of digestibility of mixed fats, although in a broad sense its effects are evident. If for no other reason, it is a mistake to hydrogenate fats so completely that their digestibilities are materially affected.

Mobilization and utilization of the fatty acids. It has long been known that dietary fats are deposited in body tissues, milk, and eggs. This occurs so extensively that it can hardly be said that any fat is characteristic of a species, unless it be that synthesized from non-fat precursors which McAmis, Anderson and Mendel called "physiological" or "synthetic" fat (133). The appearance of dietary fat in milk was demonstrated by Stohmann in 1869 (180); in body fat by Lebedeff in 1882 (112); in the fetus by Thiemich in 1905 (184); and in eggs by Henriques and Hansen (84) in 1903. The many recent papers, by furnishing more detailed information about this phenomenon, have shown that the results obtained depend upon 1, the body tissue or fluid analyzed; 2, the species of animal; 3, the kind of fatty acids used, and 4, the method of conducting the experiment. The sum of the evidence from these "tagging" experiments demonstrates the lability and rapid turnover of tissue lipids, a result confirmed and extended by the very recent use of isotopes and isomers.

The method of experimentation may largely control the absolute results. Starvation followed by refeeding brings about the most rapid change in body fats. High levels of fat in the diet produce in a short time the same result as low levels fed over a longer period. Some tissues are much more affected than others. Blood and liver lipids reflect almost immediately a change in dietary fat. More slowly the adipose tissue may change several fold in iodine number while the brain is exceptionally constant in its fatty acid content (171) (170), a stability possibly related to the inability of nervous tissue to burn fat or acetone bodies. Although phospholipids may reach a high degree of unsaturation, their percentage

change in iodine number is not equal to that which may be induced in the simple glycerides (168). Dietary fats produce changes in egg (39) (136) and milk (86) lipids which do not closely parallel the changes in depot fat (163) (93). This selective utilization of fatty acids varies so much with the species that it may be considered a "species characteristic". Certainly it plays a large part in fixing the composition of the body lipids of the different animals on their natural diets. The pig, rat and chicken are very responsive to dietary fats, their body lipids being changed profoundly by the diet (117) (54) (2) (39) without apparent effects upon their metabolism or well-being. In contrast, the body fat of beef and sheep (90) remain fairly constant in composition regardless of diet.

In those animals which respond to dietary fats a high degree of selection of individual fatty acids takes place, some being excluded from the tissues while others are preferentially held. This preference for certain acids is based upon properties associated with chain length (molecular weight), unsaturation, or melting point. It is not essential that the fatty acid be one usually found in diets or that it be the naturally occurring isomer. Erucic acid, an uncommon member of the oleic series, was found by Munk (146) to be deposited in the body fat of dogs. McConnell and Sinclair (137) were able to introduce into the lecithin and cephalin of the rat nearly thirty per cent of elaidic acid, the high-melting trans-isomer of oleic, which Barbour (7) had already shown to be readily stored in the whole body fat (9.2 per cent) and utilized in starvation like other acids in his study of the nutritive value of hydrogenated shortenings. The eleostearic acids (conjugated isomers of linolenic) are readily incorporated into tissue lipids of the rat (143) and hen (40) and secreted unchanged in the milk of the cow (97). The conjugated isomers of linoleic acid (142) and brominated acids (4) are other unnatural forms which react in the body enough like the natural ones to make them useful as tracers, a subject recently reviewed by Bloor (14) and Chaikoff (33).

Since butter fat and coconut oil are among the best food fats it is obvious that the low molecular weight acids are readily utilized when taken as a part of a mixed diet. However, a high degree of selectivity is shown by animals in their utilization of fatty acids of different chain length. In 1882 Lebedeff (112) showed that neither butter nor tributyrin in the diet increased the volatile acids of the body fat of the dog. Since that time much work has been done to establish the fact that fatty acids with less than 10 carbons are not deposited in more than traces in rat body fat (51, 50, 42, 154, 153, 37, 98, 34, 166, 118, 114). A very sharp break occurs at this molecular weight, the records showing only traces of caprylic (154), 15 per cent of capric and 25 to 32 per cent of lauric (153) (114) acids in body fats of animals fed in comparable ways. These very short acids must be immediately degraded (166) or synthesized into the longer acids and stored. There is considerable evidence for some synthesis and deposition of the higher saturated acids by addition of two or more carbons (51) (154) (166). Thus the low molecular weight acids may affect the body composition without themselves being deposited. Immediate degradation of the major portion of these acids has been demonstrated by the use of the deutero-compounds (166) as well as by their ketogenic action even when the liver is rich in glycogen (121). A

marked rise in blood acetone bodies follows ingestion of the lower fatty acids (C_4 to C_{10}) but not the higher (C_{12} to C_{18}). Another interesting observation is that caproic and butyric acids do not prevent the rise of blood sugar and the loss of galactose from the urine of rats on a skim milk diet while the higher fatty acids and many common oils bring about the normal utilization of galactose (165).

Body fats of higher animals made by synthesis from carbohydrate and protein are comprised chiefly of oleic, palmitic and stearic acids. Usually about 95 per cent of the acids have 16 or 18 carbons (54) (88) (116). The strong tendency for the maintenance of this high percentage of these two groups is well demonstrated by the work of Hilditch and Longenecker (87) in which the body fat of milk-fed rats is compared with the milk fat. Of 22.8 molar per cent of C_4 to C_{12} acids in the butter only 1.4 molar per cent was found in the body fat. There was a good storage of myristic acid. By a forced feeding of large percentages of the 10 and 12 carbon acids it is possible to have them deposited in quantity and upon starvation they are readily utilized. In fact Longenecker (114) finds a greater rate of loss of lauric than other acids from depot fats very rich in it. This might be interpreted as selective utilization but, as he points out, it may be determined by the glyceride structure. There is very little evidence for much selective use of the ordinary depot fatty acids on starvation of pigs, rats, sheep and other animals although small differences have been regularly observed (89) (90) (115). As Hilditch and co-workers point out, the general distribution of the fatty acids in mixed glycerides militates against use of a single acid if it be assumed that the whole glyceride must be utilized as a unit.

Unsaturation of a fatty acid exerts a marked effect upon its deposition. The higher animal, which is incapable of synthesizing more than traces of the highly unsaturated acids, except arachidonic, may preferentially retain a large proportion of those present in the diet (53) (2). Much of the linoleic acid of the feed is retained in the body of the pig (53). This acid is considered largely responsible for the softening of lards. When 60 per cent of the calories of the diet come from fat the iodine number of the body fat of rats follows closely that of highly unsaturated oils (2). When more saturated fats, lard and Crisco are fed there is a selective retention of the unsaturated acids which makes the body fat more unsaturated than the dietary fat. Exceptional in its effects is cottonseed oil which, upon inclusion in the diet, raises the melting point of hog lard (55) and of cow's milk fat (49).

The very highly unsaturated acids of fish oils are avidly held by certain tissues, a property which probably led to the theory of desaturation (167, 169, 168, 86). The phospholipids, especially sensitive to these acids, will show a marked change in fatty acid content with the inclusion of only a trace (50 mgm. per day) of cod liver oil in the diet of the rat (168). The change takes place quickly, being complete within 10 to 15 days (169). Their affinity for unsaturated acids is so great that phospholipids pick them up from coconut oil or butter diets which markedly lower the iodine number of the neutral glycerides (167). Linolenic acid does not seem to be so readily deposited in the rat (167), nor is it secreted in appreciable amounts by the mammary gland of the cow which does transfer much of the fish

oil acids into the butter (86). It is, however, readily deposited in egg yolk as are the acids from sperm oil (39) (40). In general, saturated acids in the diet do not so markedly affect the composition of body fats, eggs or milk. Barbour (8) concluded that, possibly due to limits of digestibility, the saturated acids of rat body fats cannot be raised above the "normal" of about 25 per cent. Cruickshank (39) also found relatively little effect of ingested saturated acids on egg fat. It seems that this rule should be applied only to the longer chain acids. When butter, coconut oil or the C_{12} or C_{14} acids themselves are fed the effects are very definite. Fasted rats, fed a diet rich in coconut oil, may deposit as much as 72.5 molar per cent of saturated acids in the depot fat (118).

In addition to being deposited or immediately oxidized, ingested fatty acids may be saturated (hydrogenated), (143) (40) (119) (166), desaturated, partially degraded to normal acids with fewer carbons (86) (114) or lengthened to a higher molecular weight (51). All of these changes, postulated from analytical data, have been given direct proof by the use of deuterium. Desaturation of stearic and palmitic acids to oleic and palmitoleic, respectively, has been demonstrated but linoleic and linolenic are not formed in this way by the animal. Nor do they take up deuterium from the heavy water of body fluids, proof that they are not synthesized by the rat or mouse but must be derived from the diet.

The essential fatty acids. This subject, recently reviewed in great detail by one of us (24), will be treated more briefly here and brought up to date by the addition of some new references. To quote from that review: "The term essential fatty acids was introduced in 1930 (29) and since that time has been used rather loosely to designate a group of unsaturated acids which will bring about a renewal of growth of rats which have reached a plateau on a low fat diet. Strictly speaking only one of these is essential. If linoleic acid is added to the deficient diet, growth will be renewed".

A large number of purified fatty acids have been tested in this way in different laboratories and it has been demonstrated that they differ in their effects upon the animal. Exclusion of fat from the diet leads to 1, development of scaly skin and caudal necrosis; 2, retardation of growth; 3, kidney lesions and hematuria; and 4, early death. Males become sterile and females produce poor litters and fail in lactation. High respiratory quotients, high metabolic rates and high water consumption have been observed and histological changes in various tissues described.

Of the above effects growth response and skin cure seem to be the best measures of effectiveness of oils and fatty acids. In some instances (62) (187) the skin is only mildly affected but this is probably due to unusually high humidity in the colony (24). The effects upon the oestrus cycle are irregular (187) and in young animals may not show up at all (156). Those acids which have been reported to have some curative effect upon fat deficient rats are linoleic, linolenic, arachidonic, decosahexenoic and hexahydroxystearic. In addition, linoleyl alcohol is useful. All other fatty acids and isomers of the above compounds have been reported negative.

Linoleic, arachidonic and linolenic acids are the only ones which have been

extensively worked with. Quantitative comparisons of their growth effects, made in different laboratories, are not in close agreement but it is clear that linoleic and arachidonic are much alike while linolenic is inferior. This acid produces some growth but does not cure the skin, reduce water consumption to normal or improve lactation (24) (156). Decosahexenoic acid and the mixed esters of cod liver oil react in a similar way and it can be said that linoleic and arachidonic acids are highly specific in their effects upon the skin.

The rat is the only species studied sufficiently to establish the daily requirement for linoleic acid. Prophylactic doses of 20 mgm. gave optimum growth in young rats (128) (28); 23 mgm. gave good growth and reproduction (124); but for optimum lactation 100 mgm. is recommended by Quackenbush, Kummerow and Steenbock (156) because the performance of their rats was superior to that reported by earlier workers. In curative experiments the higher levels of 50 to 100 mgm. daily seem distinctly superior (187) (27) (30) and it must be concluded that the lower requirements for early growth of young well-fed rats (92) is due to the stored linoleic acid which is limited by the diet of the mothers (25) (158) (157). This effect is so pronounced that the average longevity of rats on a fat deficient diet may be increased from 4 months to 14 months by changing the mothers from coconut oil to corn oil (25). Also, the iodine number of the carcass fats of weanlings may be reduced from 134 to 94 (158) by changing the mothers from stock diet to a diet made of potato meal, casein and salts.

Very few experiments have been done on the essential fatty acid requirement of other species. The goat, cow, chicken and pig have been kept for short periods on diets low in fat without the development of outward signs of the deficiency. However, the mouse responds to a lack of linoleic acid (193) very much as the rat does except that in combined pyridoxine and linoleic acid deficiency it declines and dies before developing either scaly skin or acrodynia (67). When reared on a simplified low fat diet similar to that used for an experiment with man (21) dogs develop scaly skin which is curable with fats (81). The iodine number of the blood lipids drops about 25 per cent and rises as the cure takes place.

Little is known about the requirements of man for unsaturated fatty acids. Two infants maintained by Von Gröer on a diet very low in fat grew fairly well but one developed a generalized eczema (189). Of three infants kept on a fat-free diet by Holt and co-workers (96) one developed an eczema which was cured by feeding fat. In two infants maintained at the University of Minnesota Hospital for 10 weeks on a diet extremely low in fat there was a moderate decrease in the iodine number of the serum lipids. An adult human subject maintained for six months on a diet which produces typical fat deficiency symptoms in rats felt no ill effects but he lost weight, suffered a change in respiratory metabolism and a marked fall in the arachidonic and linoleic acid content of the serum lipids (21).

The above effects parallel closely the changes in rats suffering linoleic acid deficiency. A distinct fall in blood iodine numbers accompanies the development of scaly skin (80). Assuming an analogy between rat scaly skin and simple infantile eczema Hansen and co-workers (79) began a study of eczematous human subjects, in whom they have found a distinctly lower blood content of

arachidonic and linoleic acids (20). In infants with uncomplicated eczema the average fall in serum lipid iodine number is about 25 per cent (79). When corn oil, lard or other unsaturated fats are fed most of the eczemas are cured or greatly alleviated. About 50 per cent of hospitalized adult patients with subnormal serum fatty acid iodine numbers showed distinct improvement of their skin when treated with lard by Finnerud, Kesler and Wiese (66). Although the results with human subjects are not as uniform as with experimental animals under controlled conditions, it seems clear that many people are receiving less than their optimal requirement of linoleic acid. From a survey of the national dietary it has been estimated that linoleic acid constitutes about one per cent of the average American food. It is likely that many people consume less than this amount, which has been suggested by some as the requirement for rats. It is not surprising, therefore, that there are individuals who, because of low intake or abnormally high requirement, respond to additional unsaturated fatty acids in their diet.

That the most marked human response should be in the skin falls in line with the view that linoleic acid is highly specific for that tissue. The first symptom of its deficiency is scales on the feet of young rats which may appear within 10 days and before growth has been measurably affected. The thicker and more differentiated skin of fat-deficient rats has been recently described by camera lucida drawings (195). Possibly the most striking effect of linoleic acid on the skin is produced by a combined deficiency of pyridoxine and fats. Severe acrodynia is produced which can be completely eliminated by the feeding of linoleic acid. When fed at sub-optimal levels pantothenic acid and linoleic acid supplement each other but only linoleic acid can alone cure all skin symptoms (159). In an attempt to explain these inter-relations the body lipids of the rats used in the above experiments were analyzed (158). Some differences were found in the fat content and iodine numbers of the groups but there is no evidence that pyridoxine or other B-factors bring about a synthesis of linoleic acid.

The ready mobilization of the unsaturated fatty acids is shown by the work of Smedley-MacLean, Hume and Nunn (175) (174) (176) who find that the arachidonic acid of subcutaneous tissue is rapidly moved to the growing liver and developing tumors. Tumor growth was not retarded by limiting the intake of unsaturated acids.

A new specific function of linoleic has been described by Engel (56). With synthetic vitamin supplements and a fat-free diet, choline had its optimum lipotropic effect only when inositol, pyridoxine and linoleic acid were all present. One-tenth milliliter of corn oil daily reduced the liver fat to half in one experiment. The author postulates a mutual influence of these factors in the mobilization of fat, although it is known that linoleic acid deficiency does not materially decrease the rate of incorporation of labeled fatty acids into liver phospholipids (9).

The relationship between dietary fats and other nutrients. Fats markedly affect the availability and utilization of a number of dietary essentials. It is difficult to evaluate the importance in human nutrition of these effects which are readily demonstrated with laboratory animals. In certain cases, particularly when oxi-

dized or rancid fats come into intimate contact with other foods either in the dietary mixture or in the intestinal tract, the relationship of fats to other nutrients assumes an increased significance. In the following discussion an attempt has been made to bring together some of the pertinent information on the effects that both fresh and rancid fats have on other dietary substances.

Vitamin A. It has long been recognized that different fats have a profound effect upon the biological efficacy of vitamin A and its precursor, carotene. Only one year before Moore's (144) discovery that carotene is converted into vitamin A in the animal body, Duliere, Morton and Drummond (45) found that carotene which was dissolved in ethyl oleate was biologically inactive and reported that carotene did not exhibit vitamin A activity. Even before this time it had been observed that vitamin A was poorly utilized when administered in mineral oil (46, 31, 140) and following this that the utilization of carotene was even more affected (47). Natural fats were shown to have varying effects upon carotene utilization and in 1935 the Medical Research Council concluded that in vitamin A assays the standard β carotene was more efficacious if dissolved and administered in coconut oil or certain samples of arachis oil than when given in olive oil, hardened cottonseed oil, ethyl laurate, or liquid paraffin (139, 109).

It is probable that at least two separate factors are responsible for the diverse effects of fats on vitamin A and carotene utilization. These are 1, absorption, and 2, oxidative destruction. Largely through the well known work of Drummond, Bell and Palmer (44), and Greaves and Schmidt (75), it is now known that to some extent both carotene and vitamin A follow the general pathway of fat absorption. Furthermore it has been shown by Ahmad (1) and Mulder and Kelly (145) among others that dietary fat is necessary for the normal absorption of vitamin A and that with amounts up to at least 10 per cent of the total weight of the diet, the apparent absorption of the vitamin increases.

It is probable that the loss of biological potency, apparent when the vitamin or its precursor is administered in various natural fats, is largely due to oxidative destruction before absorption takes place. This oxidation might be brought about by simple diffusion of atmospheric oxygen through a solvent which in itself neither accelerates nor inhibits the reaction. However, the formation of prooxidants which increase the rate of carotene destruction is of more serious consequence. Baumann and Steenbock (11) found that carotene dissolved in ether, chloroform, cyclohexane, acetone, carbon disulfide, toluene and pyridine, and kept at 4°C. was more than 75 per cent destroyed in 5 months while other solvents such as benzene and petroleum ether were less destructive. Ether, the solvent which caused the greatest loss, readily becomes contaminated with peroxides.

In the above study, hydroquinone effectively reduced carotene destruction in all of the organic solvents that were employed. In 1931 Olcovitch and Mattill (148) came to the conclusion that the stability of carotene in solution is dependent upon the nature and the amount of antioxidant present. The importance of antioxidants was further stressed by Shrewsbury and Kraybill (173) who found that if butter fat was treated with charcoal the vitamin A and carotene, together with natural antioxidants, were destroyed and furthermore that if carotene was

added to this charcoal treated butter fat and immediately fed to rats an appreciable part of its potency was lost. If hydroquinone was also added, the carotene was protected against *in vitro* oxidation but it still lost a part of its biological activity (106). A number of results have been published on the biological potency of carotene dissolved in different fats and in many of these no correlation between *in vitro* oxidation and biological potency could be observed. Sherman (172) clarified some of the discrepancies in earlier publications by showing that there are real differences in vitamin A potency dependent upon the fat in which it is administered, but that this effect is observed to a noticeable extent only when the basal diet is fat-free except for the essential fatty acids.

Another demonstration of the influence of antioxidants on vitamin A potency was recently presented by Hickman, Harris, and Woodside (85) who used the U. S. P. vitamin A assay method with olive oil as the specified vegetable oil in the basal diet and studied the effect of added vitamin E (molecular distillate containing 40 per cent mixed tocopherols) on the growth response to fixed quantities of three different forms of vitamin A (crystalline alcohol, a crystalline acetate, and mixed carotenes). With all three substances the addition of sufficient vitamin E markedly increased growth. In the case of carotene, a dosage of the vitamin that was insufficient to support life if given alone, induced good growth when fed with sufficient vitamin E.

It was observed that the tocopherols were effective only if administered simultaneously with the vitamin preparations. This would indicate that they protected the vitamin A from oxidative destruction while in the intestinal tract. Baumann, Riising and Steenbock (10) showed that when a halibut liver oil concentrate dissolved in cottonseed oil was administered to rats absorption and storage of vitamin A in the liver took place to a large extent within 6 hours, but losses due to destruction in the intestinal tract were large during this time. Fecal losses were small so that faulty absorption could not account for the estimated intestinal loss. If intestinal destruction of vitamin A is large, but is prevented by vitamin E, hydroquinone which is a much more potent antioxidant than vitamin E might be expected to have a greater effect. However, as indicated by Kraybill and Shrewsbury (106) and Quackenbush, Cox and Steenbock (155), this is not the case. The latter investigators using both a distillate of soybean oil and α-tocopherol together with carotene dissolved in ethyl linolate (peroxide value = 0) have come to the conclusion that hydroquinone, being a water soluble compound, is extracted from carotene solutions in the intestinal tract and therefore cannot exert any antioxidant action at this site. On the other hand α-tocopherol being fat soluble remains with the carotene and protects against oxidation during its passage through the intestinal tract.

With the development of rancidity *in vitro*, natural antioxidants are used up and carotene and vitamin A are destroyed. The substance in rancid fats that causes this destruction is still unknown. Lease, Lease, Weber and Steenbock (111) in an extensive study on this subject have come to the following conclusions: Vitamin A is not destroyed by oxidized glycerol, glyceric acid, glyceraldehyde, allyl alcohol, straight chain aldehydes, methyl ketones of 7 to 12 carbons

or decomposition products formed by the commercial hydrogenation of fats. The components of rancid fats which cause destruction of vitamin A are not removed by steam distillation nor by extraction with alcohol. From the vast literature that has now accumulated on this subject of *in vitro* vitamin A oxidation it would appear that loss of activity is associated with the intense peroxide formation that follows the termination of the induction period of the fat in which the vitamin is dissolved. Sumner (181) has illustrated this relationship between rate of peroxidation and carotene destruction by using active lipoxidase preparations. It is his conclusion that intermediates of fatty acid peroxidation are the active agents involved, but not the peroxides themselves.

Vitamin D. It was early pointed out by Zucker and Barnett (196) that dietary fat exerts some antirachitic action and this observation has been amply substantiated by more recent investigations (104) (105) (138) (15) (76) (103) (100) (101) (17). The antirachitic effect of fat in the diet is relatively small but consistent, and is dependent upon the amount of fat present. For example, Knudson and Floody (103) found that with 0.025 mgm. of crystalline vitamin D_2 dissolved in propylene glycol and added to 40 grams of a rickets producing basal diet, the extent of healing was about 3 times greater if 5 per cent hydrogenated vegetable oil was included. With 10 per cent fat the vitamin D was less effective and 20 per cent reduced the healing to the approximate level of the fat-free basal diet.

Explanations of this antirachitic effect of dietary fat have been largely concerned with the influence that fat might have on calcium and phosphorus absorption. It is known that in sprue, celiac disease and in other types of profound steatorrhea, calcium absorption is impaired, the unabsorbed calcium being excreted in the feces, for the most part, as calcium soaps. It has also been observed that when the fat content of the diet is greatly increased the amount of calcium soaps in the feces increases (38) (5) (160) (13). However, it has been often reported that small amounts of fat in the diet have a beneficial effect upon calcium absorption (94) (95) (19) (70), but it is not fully agreed that fat increases the absorption and retention of calcium or the calcification of bones (17) under all conditions. It would appear that both calcium absorption and calcification are more definitely enhanced by dietary fat in the rachitic animal than in the normal.

In an attempt to explain the manner in which vitamin D-free fats influence calcification Jones (101) studied the effect of a number of substances on the acidity of the intestinal contents and on the calcification of the bones of rachitic rats. In agreement with the work of others it was found that fat (10 per cent lard or oleic acid) decreased the pH of the lower portion of the small intestine at the same time showing a definite antirachitic effect. However, lactose also increased the acidity of the lower intestinal tract, but did not exhibit any antirachitic action. Dibasic phosphate protected the rats against rickets without changing the pH of the intestinal tract. Although these experiments do not completely rule out intestinal acidity changes as a factor involved they do point out the obscurity of the mechanism of the fat effect on calcification.

Vitamin E. Early in the development of the knowledge concerning the physi-

ology and chemistry of vitamin E it was pointed out that amounts of wheat germ oil which added to a fat-free diet were sufficient to relieve sterility in rats became ineffective if lard, crisco, or oleic acid were included in the diet (60). The explanation for this effect of fat on vitamin E activity, simultaneously presented by Mattill (129), and Evans and Burr (59), was that oxidative changes which accompany the development of rancidity in unsaturated fats destroy the vitamin. Macomber (125) added 5 per cent wheat germ to a diet containing 20 per cent lard and found the diet to be deficient in vitamin E.

Goettsch and Pappenheimer (73) first pointed out that rabbits and guinea pigs developed a characteristic muscular dystrophy when fed diets that were treated with ferric chloride and contained lard and cod liver oil. The ferric chloride treatment was known to destroy vitamin E (190) and the lard and cod liver oil additions seemed to aggravate this deficiency symptom. Madsen (126) found that muscular dystrophy could be produced by a vitamin E-free diet containing a cod liver oil concentrate without lard being present. The addition of cottonseed oil afforded some protection, but if cod liver oil in its natural form was included, cottonseed oil was without effect. In view of the fact that muscular dystrophy can be brought about by the ingestion of cod liver oil concentrates as well as by the natural cod liver oil it is interesting to note that Evans and Burr (59) had found that the vitamin E destroying material of oxidized lard could be concentrated in the unsaponifiable fraction.

Although it is not fully agreed that the sole reason for the development of muscular dystrophy in certain animals fed cod liver oil is through a destruction of vitamin E, there is an accumulation of evidence that the dystrophy can be prevented by the appropriate administration of α-tocopherol (123) (122) (57) (130). It is the opinion of Mattill and Golumbic that there is no difference between the muscular dystrophy of cod liver oil and that produced by lack of vitamin E. If this is true it is probable that the influence of vitamin E-free fat in the diet in protecting against or aggravating the onset of deficiency symptoms is entirely due to an anti-oxidant protection of the vitamin or a pro-oxidant aid in its destruction. Either might take place before or after the ingestion of the dietary mixture. A complete review of the studies on muscular dystrophy has just been published by Pappenheimer (150) in this journal.

Vitamins of the B complex. The very striking sparing action that dietary fat has on thiamin has long been recognized. While the excellent reviews that have been written on this subject, especially those of Williams and Spies (194) and Cowgill (36), adequately bring the literature up to recent years, a few contributions should be discussed.

Stern, Arnold and Elvehjem (179) in a study of the B_1 sparing action of fat, isocalorically replaced the carbohydrate of a B_1-deficient diet with fat and found that rats which had developed polyneuritis through previous subjection to a low fat, thiamin-free regimen grew well, exhibited no signs of polyneuritis, and the females had normal estrous cycles. The symptoms of polyneuritis which the rats had when the fat diet was started did not completely subside for about 1 week although growth during this time was good. These authors point out

that since thiamin administration causes the disappearance of polyneuritic symptoms within 4 to 6 hours, fats must act in a manner independent of thiamin. Salmon and Goodman (164) had previously reported that a diet containing 40 per cent fat, but very low in thiamin, did not prevent the occurrence of polyneuritis in rats. Stern, Arnold and Elvehjem offer the suggestion that perhaps the diet used by Salmon and Goodman was low in some dietary factors other than thiamin for when the autoclaved yeast content of a high fat diet was increased symptoms of polyneuritis disappeared. It has been well established by the use of autoclaved and synthetic fats that the sparing action is not due to the presence of thiamin. Melnick and Field (141) have carried out chemical determinations for thiamin in fats, with negative results. Furthermore, Kemmerer and Steenbock (102) found no increase in the amount of thiamin in body tissues when thiamin deficient rats were fed high fat diets, and Cahill (32) has found that in humans ingesting a diet with a constant thiamin content, the addition of fat had no influence on the amount of thiamin excreted in the urine. These results together with recent information on the participation of thiamin in the carboxylase system indicate that fat spares thiamin by altering metabolism in such a way as to circumvent its need. An observation that could be interpreted as a confirmation of this idea has been presented by Banerji (6) who found that the ingestion of a high fat diet not only alleviated the severity of a thiamin deficiency bradycardia, but diminished the excretion of bisulfite-binding substances in the urine.

Evans, Lepkovsky and Murphy (65) noticed an apparent aggravating effect of fat upon the vitamin G requirment of rats. More recently Mannering, Lipton and Elvehjem (127) have shown that fat at a level of 25 and 40 per cent of the diet, markedly increased the riboflavin requirement of the rat, while with the dog the level of dietary fat has no influence on the need for this vitamin (152).

Dietary fat seems to have some sparing action on the pantothenic acid (188) and biotin (147) needs of the rat. Furthermore certain fats (corn oil) give a much greater protection from egg white disease than others (Crisco), an observation that might be associated with their content of the essential fatty acids (120).

Rancid fat. In the foregoing discussion some mention has been made of the deleterious effects of oxidized fat in the diet, particularly in the destruction of vitamins A and E. Serious as the effect on these two vitamins is, the detrimental properties of rancid fat are much more extensive.

The stability of all the essential nutrients to oxidized fat is not known, but on the basis of chemical structure and properties, the following substances might be considered capable of destruction by such pro-oxidants: Vitamin A, carotene, vitamin D, vitamin E, pantothenic acid, pyridoxine, biotin, ascorbic acid, and linoleic acid. There is a serious lack of information concerning the possible destruction of many of these substances by oxidized fat, but that information which is available will be discussed here.

It was pointed out by Mattill and Golumbic (130) that many investigators working with E deficient animals have occasionally encountered dermatitis in

older animals and caudal necrosis in the new-born. The dermatitis is sometimes relieved by the administration of wheat germ oil. As the wheat germ oil *per se* is possibly not responsible for the cure of dermatitis symptoms it may have protected other factors by its antioxidant properties. Neither pantothenic acid nor pyridoxine have been studied, but at least one of the dermatitis preventing vitamins, biotin, has been shown to be destroyed by organic peroxides (23) and by products produced in rancid fats (151a).

While the most important factor concerned in the oxidation of vitamin D in animal foods seems to be the amount of exposed surface area, rancid fats are also of importance in its destruction (71).

Ascorbic acid, which is known to act as a fat antioxidant, is not appreciably destroyed during the induction period (74). Experiments from the authors' laboratory indicate that when pure ascorbic acid is suspended in highly rancidified fat it is not oxidized.

The peroxidation of unsaturated fats naturally destroys linoleic acid. Feeding of such oxidized fats has been shown to result in the development of skin changes in rats (192) and dogs (191), suggestive of linoleic acid deficiency, but in a much more severe form than that ordinarily produced by exclusion of the essential fatty acids.

The presence of rancid fat in the diet has effects other than in destroying essential nutrients. György *et al.* (78) studied a diet of the following composition: Casein 6, cornstarch 50, sucrose 22, cod liver oil 2, salts 4, and linoleic acid (crude) 16 with daily supplements of thiamin, riboflavin, pyridoxine, and pantothenic acid. Within 3 to 4 weeks standing at room temperature the linoleic acid was almost completely destroyed by oxidation. If butter yellow (N.N.-dimethylaminoazobenzene) was incorporated in the diet at a level of 0.06 per cent, it was destroyed by the oxidizing linoleic acid. These authors are of the opinion that such substances as Crisco, butterfat, or rice which have a procarcinogenic effect when given together with butter yellow do so by stabilizing the carcinogen either by reducing the unsaturated acid content of the diet or through an antioxidant effect. However, this can not be the explanation for the enhancing effect of dietary fat on tumor production by skin application of 20-methylcholanthrene for in this case rancidified fat is equally effective (110). Of striking interest in the study by György *et al.* was the observation that the diet containing linoleic acid was very toxic. Rats fed this regimen either with or without butter yellow, lost weight, developed an anemia of the secondary type, a leucopenia, and were subject to pediculosis. These symptoms could be prevented by the daily addition of yeast to the diet. The toxicity was apparently due to oxidation products of the unsaturated fatty acid.

In this laboratory loss of weight and death have been noted when a balanced diet containing casein, glucose, lard, salt mixture, and pure B vitamins together with cod liver oil and wheat germ oil was fed to rats. The toxicity of this diet was related to the development of rancidity, but as cod liver oil has been included in the diet in the same manner as in the György study it was thought probable that a deficiency of vitamin A had developed. Yeast was also found

protective, but on further study it was found that the yeast exerted a strong antioxidant effect which protected the lard from oxidation. When cod liver oil was fed separately and oxidation of the fat was prevented by refrigeration no toxic symptoms were manifested, but if the lard was rancidified to a peroxide value of 200 to 400 milli-equivalents per 1000 grams and again the cod liver oil fed separately loss of weight, decreased hemoglobin, and red and white blood cell count resulted. The livers of rats showing these symptoms had normal amounts of vitamin A, so the toxicity must have been due to other causes.

The anemia that has been reported by György *et al.* and also observed in this laboratory might be due to some indirect cause. It is of interest that Haurowitz, Schwerin, and Yenson (83) have found that in the well known catalytic effect of hemin and hemoglobin on the oxidation of unsaturated fatty acids, both pigments are completely destroyed. In this reaction inorganic iron is released, but neither porphyrins nor bile pigment derivatives are found. If there is any physiological significance in these observations, the oxidation products of the unsaturated acids that act as pro-oxidants must be absorbed from the intestinal tract and enter the blood stream. While there is at present no definite answer to this point there are reports that have some bearing. Irwin *et al.* (99) report that hydrogenated cottonseed oils having peroxide values of 107.6 and 80.1 and melting points of 45.3° and 45.6°C. respectively are absorbed to the extent of 90–92 per cent within 12 hours. Unpublished results from this laboratory show that when highly oxidized fat is ingested by rats the keeping time of the animals body fat is decreased. This does not necessarily mean that pro-oxidants are deposited in the adipose tissues for the peroxide value of the freshly rendered body fat is zero. Furthermore as was mentioned above the vitamin A stores in the liver are maintained at a normal level. The decreased keeping time of adipose fat after the ingestion of rancid fat could be due to the complete destruction of all dietary antioxidants. Under normal conditions these antioxidants might be absorbed and help stabilize the body fat. It is very likely that some products of oxidation are absorbed from the intestinal tract. For example, heptyl aldehyde (35) which is known to occur in small amounts in rancid fat, is readily absorbed and enters the body tissues. If harmful products of rancidification are absorbed they must do so to only a small extent for parenterally administered these substances exhibit a toxicity far beyond that observed following oral ingestion (48).

From the foregoing discussion it can be seen that the presence of highly oxidized fats accelerates the destruction of a large variety of food factors. While most of the detrimental effects of ingestion are probably due to induced nutritional deficiencies the possibility remains that some activity is directly manifested in the body tissues. The inadvertent addition of oxidized fat to a diet mixture, the development of fat oxidation in the diet due to lack of proper stabilization and the possibility of oxidative changes in the intestinal tract must be given serious consideration in practical nutrition as well as in the theoretical interpretations of experimental studies.

The value of dietary fat as measured by growth and reproductive performance. Many experiments have demonstrated that growth, lactation, reproductive

performance and other metabolic functions are altered by the consumption of fats. The results obtained are not always readily correlated with the composition of the fats and their expected digestibility, mobilization and retention, essential fatty acid content or effects on other nutrients. From the preceding discussions it is conceivable that fats of widely different fatty acid contents might have very different nutritive values. The cases to be cited below may be considered typical of the scores in the literature which record effects of quantity or quality of dietary fats.

Nearly all experiments have shown that the inclusion of fresh, palatable and digestible natural fats improves the diet in some way. Infants on fat-free diets develop loose, mushy, fermentative stools (96). Calves do not grow as well on skim milk supplemented with fat-soluble vitamins as on whole milk (77) or reconstituted milk into which butter oil, lard or beef tallow had been homogenized. On low fat the average daily gain was 1.07 pounds as compared with 1.43 pounds for the whole milk group. Cows (131) (72) and goats (12) produce less milk and butter fat on low fat diets. On a ration containing only 0.45 per cent ether extract decreases in milk yield of goats ranged from 25 to 55 per cent and the butter fat yield dropped 35 to 70 per cent. With cows the effects have not been so striking but a summary of experiments done by Maynard and co-workers over a period of 12 years has demonstrated that diets containing about 6 or 7 per cent of fat increase yields of both milk and butter fat as compared with diets containing less than 4 per cent fat. The depression of milk and butter fat yields, very marked when cod liver oil or menhaden oil is fed to cows (134) (22), is slight or lacking on the inclusion of shark liver oil (134) (161) (43), salmon oil (134), or unsaturated plant oils like corn oil (183) in the diet. In fact some of the above increases were obtained with the highly unsaturated soybean oil. Growing chicks (162) have been kept 14 weeks on a grain ration so low in fat that the iodine number of the body fat was greatly reduced without retarding the growth significantly although the average weight of both males and females on the low-fat ration was already slightly low at the end of this short (14 week) period. When laying hens were put on a diet containing less than 0.1 per cent fat there was a tendency toward a lower and less sustained egg production (163).

Many experiments with rats are not useful to this discussion because proper low-fat diets were not used as controls or growth and reproductive performance was not recorded. However, there are a number of experiments which show definite improvement on the inclusion of some fat in the diet (in excess of the essential fatty acid requirement). Diets containing only the fat in crude casein and yeast and supplemented by 2 or 3 drops of cod liver oil do not produce maximum growth, ovulation or lactation (61) (62) (63) (64). Twenty or twenty-five per cent of lard, butter, or cottonseed oil is better than five or ten per cent of lard. It is not certain that the low-fat diets were always adequate in the essential fatty acids. With higher levels of fat it has been found that diets containing 30 to 55 per cent of different fats produce greater weight gains in young rats than does a 5 per cent diet (91). In this laboratory at the present time there are groups of rats on diets containing 20 per cent of several fats. All are superior in growth and lactation to controls receiving 10 drops of corn oil (Mazola) daily. The

average weight of the high fat animals is 13 per cent above that of the low fat group. It is not known in any of these cases whether the increased weight is due entirely to obesity. However, with mixed diets of natural foods containing 4.50 and 9.29 per cent of fat, it has been recently shown that rat mothers with the higher fat intake show improved lactation and that the young at 17 days of age contain more dry matter, more fat and more protein (132). There are, however, experiments in which rats receiving up to 55 per cent of their diet as fat did not grow more in the first six weeks than the controls on sucrose (164). There is also the experience of those working with fat deficiency that fat-free diets supplemented daily with only 25–100 mgm. of linoleic acid produce fair growth and lactation (124) (156). In the first case (124) the performance is definitely below optimum and in the second (156), while it is said to compare favorably with that of the stock colony, no exact comparison of weaning weights is given. There is a preponderance of evidence that several species respond favorably to added dietary fat. Since all of these species have great powers of fat synthesis from carbohydrate (except for certain unsaturated acids) and are capable of utilizing either of these food materials for work with almost equal efficiency (107) it is not surprising that the differences are small. Nevertheless for optimal nutrition fat should furnish a considerable proportion of the calories.

Large differences have been reported in the comparative nutritive value of different lipids, both natural and synthetic. The outstanding feature of these experiments is the irregularity of results from different laboratories and even within one laboratory. Most of the work has been done with rats on a basal diet to which have been added equal amounts of the fats under test. Usually ad libitum feeding has been allowed and the overall growth effects recorded. In some cases, however, the gain per calorie intake has been calculated or the food consumption limited and the gain per unit of fat recorded. Since there is little difference in the composition of different batches of commercial fats, it seems likely that the method of feeding or unrecognized effects of rancidity on the rest of the diet may account for the lack of uniformity. Lacking space for a detailed discussion here of each experiment with comments on possible factors affecting the results, only a few of the older experiments will be given along with the most recent ones.

From the work of Ozaki (149), Eckstein (51) (52), Powell (154) (153), Davis (42), Barbour (8), Evans and Lepkovsky (62) (63) (64) (113), Cox (37), and Salmon and Goodman (164) with synthetic esters and salts of the fatty acids it is evident that the comparative growth promoting value of these materials depends upon the form in which they are fed, the percentage in the diet, and the adequacy of the rest of the diet. Even as much as 60 per cent of the diet may be composed of the methyl, ethyl, glycerol or propylene glycol ester of mixed fatty acids of lard without ill effects (113), only the ethylene glycol ester being toxic. Soaps may also be fed successfully at lower levels. In general the glyceryl esters seem best. This is especially true when single fatty acids make up as much as 75 or 80 per cent of the calories. For example, ethyl laurate is very toxic at this level (37) while laurin is tolerated and promotes growth (164). Butyrin and

ethyl butyrate are highly toxic as contrasted with the esters of acetic or caproic acids which allow fairly good growth. There is a sharp distinction between the 4-carbon acid and its neighboring homologues. Its ill effect on growth is due not only to a depression of appetite resulting in starvation but to an injurious effect upon tissues (42). Toxic effects of the other single fatty acids become evident only when they are fed at very high levels and the admixture of other acids eliminates the difficulty. In practically all cases the natural fats are superior to glycerides resynthesized from single or mixed fatty acids. In fact, only when the best of natural fats are added is the performance better than on a low fat-high carbohydrate diet. The addition of 20 per cent or more of synthetic esters of single or mixed fatty acids usually results in growth inferior to that on the basal diet (64) (164).

The order of relative efficiency of different fats may be reversed by a change of level in the diet as well as by a partial deficiency in the diet such as thiamin (64) (164), or vitamin A (24). Ad libitum feeding may reverse the order as compared with restricted feeding.

There are many experiments which demonstrate that, with not over 30 per cent of fat in the diet, the growth of rats is good on fats ranging in composition from coconut oil to linseed oil (25) (91). When very great differences due to small amounts of fat are observed the diet should be examined carefully for some deficiency, possibly due to the action of the fat itself. The effects of different oils are not shown as strikingly with rats as with calves which fail to grow, decline, and die when liquid fats are homogenized into skim milk (77). But with the rat there is considerable evidence that some liquid fats like cottonseed or corn oil do not equal solid fats in promoting early growth (164) (25) (91), especially when they constitute a large part of the diet. Although the superiority of butter fat for calf nutrition seems established it is not so readily demonstrated with the rat. Using the method of homogenizing fats into mineralized skim milk more rapid early growth on butter fat has been found (18) (24) but the results are sometimes small and not always regular. These differences in early growth were not found by Freeman and Ivy (69) when commercial evaporated milk was compared with a filled milk containing coconut oil instead of butterfat. After three months, however, the animals receiving butterfat were larger than their controls. With simplified diets it has been found by Harris and Mosher (82) that the results can be reversed by using animals of different age, weanling rats growing more rapidly on butterfat and older rats more rapidly on coconut oil. Premature babies and twins, in whom fat utilization is defective, absorb olive oil and soybean oil far more completely than butter, with a resulting improvement in growth and nutritive condition (185). Some early experiments with solid diets indicate a superior value of butter for lactation (61), but more recently Sure (182) has reported no differences in lactation performance on diets containing 15 per cent of several fats, including butter. Recently Boer and Jansen (16) have postulated a new growth promoting fatty acid in butter. However, Euler, Euler and Säberg (58) report better growth of rats on margarine. In our laboratory at the present time there are six groups of rats which show that at the 20 per cent level

butter is just equal to several other fats. Some older experiments, in which very high levels of fats were used (55 and 60 per cent of the diet), show distinctly superior growth on other fats as compared with butter (164) (62). If each of the other commercial fats is reviewed, conflicts in results will be found similar to those mentioned above for butter. The answer seems to lie in the variability of the products used by the workers as well as in the differences in methods of experimentation. The former effect is well illustrated by the recent paper of Hoagland and Snider (92) in which several lards are compared with several hardened vegetable and animal shortenings. The average growth rate of rats for all the lards was essentially the same as for all the shortenings. However, the lards differ significantly among themselves. Until all workers use exactly the same materials and conduct the experiments under identical conditions, conflicting results will continue to appear. From the explanation of these differences will come a more complete knowledge of the rôle of fat in the diet.

SUMMARY

There are ample reasons for recommending that the fat intake be not reduced much below the normal established by habit. To give the best results added fats must be fresh. Rancidity renders them unpalatable, destructive to other vital foods and possibly slightly toxic in themselves. Frequently the preservation of a mixed food is largely a matter of the prevention of fat deterioration.

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QUANTITATIVE AND QUALITATIVE VARIATIONS IN NORMAL LEUKOCYTES

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Two excellent reviews have been written dealing primarily with changes of the leukocytes in the circulating blood under different physiological conditions; in man by Garrey and Bryan (1936), and in rabbits by Cheng (1930). The reader is referred to these publications for a comprehensive consideration of the literature and summary of the status of our knowledge concerning the subject to the date of each publication. The present article was requested because some important contributions have been made since the appearance of the two reviews mentioned above. The material in recent articles has been based on studies which, in some instances were more accurate technically, due to the use of standardized apparatus and technic; the results have been treated by modern statistical methods; more important observations have been made on changes in the differential formula and on the maturity of the neutrophils as indicated by the shape of the nucleus. Furthermore, there has been a growing appreciation that under normal conditions, at short intervals, there is a spontaneous and irregular fluctuation of the total number of leukocytes and of the percentages of the different varieties of white corpuscles, which may easily be misinterpreted unless evaluated critically. Hence any changes in the leukocytes, especially when they are within the range of normal, or moderate in extent, should not be interpreted as due necessarily to any single or constant physiological or pathological influence, unless the trend is a uniform one in multiple experiments.

Recognition of the cause and extent of the physiological variations in the leukocytes is important when fluctuations in their number and variety are utilized as evidence of significant alterations either in experimental or human physiology. Undoubtedly conclusions have been reached without proper allowance for observational error, spontaneous variations, or the uncertainty of isolated determinations. In clinical medicine, decisions have been based on one or a few widely spaced leukocyte counts, with a differential formula determined by a survey of only one hundred cells and with a total disregard for their physiological variations. Since the introduction of the sulfonamide drugs, for example, there has been a tendency to regard a decrease in the white blood cells as evidence of the toxicity of the drug and hence as an indication for its discontinuance, even though the diminished total white count remains within normal limits. As a consequence, an erroneous interpretation may lead to the denial to the patient of an exceedingly valuable and sometimes life-saving therapeutic agent.

Our present knowledge indicates that variations in the white blood cell count of the circulating blood may occur as the result of the action of any one, or a combination, of the four following mechanisms:

I. Alterations in the rate of production of leukocytes. A decrease in the leuko-

cyte count may result from simple inhibition or maturation-arrest in the bone marrow, whereas an increase is dependent upon simple hyperplasia or accelerated maturation.

II. Variations which are dependent upon changes in their rate of destruction or elimination from the body.

III. Changes in the number per cubic millimeter resulting from differences in concentration of the circulating blood plasma.

IV. Redistribution of the white blood cells in the vascular channels. Evidence in recent years tends to reaffirm the earlier belief that this mechanism is the basis for many, if not all, of the physiological variations in the leukocyte count.

The above classification is based, with modifications, on the information presented by Lawrence (1941), to which reference should be made for fuller details.

Observational variations. Variations in total leukocyte counts attributable to observational error may be separated *a priori* into two classes: 1, the "error" involved in drawing conclusions relative to an entire population on the basis of data derived from observations on small random samples; 2, "blunders" introduced by defective technical methods and inaccurate apparatus. Although, in practice, one cannot disregard the influence of "blunders" in the evaluation of individual counts, it is nevertheless possible so to standardize the conditions of observation that variations introduced by the second class become negligible thereby permitting a statistical approach to the "error" of random sampling. Bryan, Chastain and Garrey (1935) point out that this error is influenced by three factors; *a*, mixing of the cells and diluting fluid; *b*, filling the counting chamber by capillarity, and *c*, settling of cells by chance on the ruled field of the counting chamber. They are able to demonstrate that the last named, chance settling of the cells, is responsible for practically all of the major variations of subsampling. It therefore becomes possible, within reasonably accurate limits, to apply the laws of a normal frequency distribution system in the statistical evaluation of total leukocyte counts, when the conditions of observation are sufficiently well standardized. These authors also point out that more accurate expression of the "error" of physiologically normal leukocyte counts is gained by the use of a scale of absolute numbers rather than percentages since the latter are partly dependent on the means of the leukocyte counts. Based on an analysis of 2508 white blood cell counts they report an average error of $\pm$ 241 $\pm$ 35 cells per cubic millimeter when 10 unit hemacytometer areas of one square millimeter each and a blood dilution of 1 to 20 are employed. The magnitude of this error did not alter appreciably at the different levels of the observed leukocyte counts as was shown by the low value of its probable error.

It has also been demonstrated by Plum (1936) that when the conditions of leukocyte enumeration are adequately controlled the accidental distribution of cells in the counting chamber becomes the only important variable. His data, obtained by empirical observation, led him to conclude that a systematic relationship exists between the magnitude of the standard deviation and the number of

cells enumerated in each determination, and that this relationship may be expressed in terms of a Poisson's series according to which the standard deviation is equal to the square root of the counted number of cells.

For a laboratory procedure in such common use as the determination of percentages of the several types of leukocytes, the differential count, the many technical methods employed and the lack of exact information concerning their respective accuracy, constitute a striking deficiency in hematologic practice. Moreover, few observers possess well-founded knowledge of the statistical error involved in the differential count when the distribution of the cells in the stained film is assumed to be entirely "accidental."

Differential counts of five entire blood films, made on slides with a cover slip spreader, and an almost complete count on a sixth, were compared by Mainland, Du Bilier and Stewart (1935) with four clinical methods of differential counting. It was found that the results obtained either by counting three sets of 100 leukocytes, using cross rows, one 3 mm. from the beginning, the second at about the middle and the third about 3 mm. from the end of the film, or by counting all the cells in the middle longitudinal row, one field in width from beginning to end of the film, agreed sufficiently closely with the total counts to be attributed to random sampling. On the other hand, more complicated procedures, such as Schilling's "meander-four-field" method and Silberstein's method, showed discrepancies greater than are usually attributable to chance. For the cross count method, with allowance for the size of the samples employed, the authors estimate a range for the neutrophil percentage, attributable to random sampling, from 7.4 per cent above the true value to 7.4 per cent below it. The minimum ranges of error for the other cell types were: lymphocytes, 6.1 per cent; monocytes, 2.6 per cent; eosinophils, 2.4 per cent; basophils, 1.0 per cent. These values, derived from small samples for which due allowance was made, should include about 99 per cent of all observations. In a later publication, Mainland, Coady and Joseph (1935) estimate the observational variation for the cross count method of differential enumeration using slide preparations. They point out two sources of variation affecting the differential count, apart from the error involved in truly random sampling. These variations are well recognized, but frequently overlooked by the laboratory worker. They consist of: 1, abnormalities of distribution of the several types of leukocytes on the film, as prepared by the slide method, so that in differential counting one is "taking a sample merely of the area of film examined, not a representative sample of the whole drop"; 2, differences between successive drops of blood and between blood obtained from more than one puncture. Their data were obtained from twenty-four healthy male students. Across each of seven blood films, prepared by the slide and cover glass method, five from successive drops from a right ear puncture and two from successive drops from a left ear puncture, made on the same occasion from each student, three sets of 100 leukocytes each were counted. The variations of these sets were greater than could be attributed to purely random sampling, indicating, therefore, that in the comparison of differential counts the methods employed must be strictly adhered to. Their technique permitted

exclusion of such factors as thickness, evenness, and length of film, and permitted the inference that the observed variations were due to irregularities of cell distribution, especially along the edges of the films. The differences observed between drops were also greater than might have been anticipated as due to random subsampling. Therefore, more accurate results may be secured by counting on films prepared from two drops of blood, than by utilizing only one drop, even though the total number of leukocytes enumerated is the same in both cases.

Although Mainland and his associates point out that the distribution of blood corpuscles, as they are counted on a film, is discontinuous and therefore not truly represented by a normal curve, they submit data justifying the assumption that for purposes of statistical calculation the distribution may be considered as symmetrical when the conditions of examination are strictly defined. On this assumption, for the 24 healthy male students, aged 19 to 28 years, whose bloods were examined in the early spring, the following figures are submitted based on a count of 2100 leukocytes for each student (table 1).

TABLE 1

	MEAN PERCENTAGE	STANDARD ERROR OF MEAN	STANDARD DEVIATION OF SERIES	COEFFICIENT OF VARIATION
Neutrophils	56.88	±1.201	±5.886	10.4
Eosinophils	2.01	±0.209	±1.023	50.9
Basophils	0.44	±0.042	±0.204	46.5
Lymphocytes	37.03	±1.221	±5.982	16.2
Monocytes	3.64	±0.168	±0.825	22.7

The unavoidable error in differential counting, or that due to the accidents of random sampling where the distribution is assumed to be normal, has been calculated by Barnett (1933) according to Bernoiulli's theorem: $SD = \sqrt{n.p.q.}$, where SD is the standard deviation, n is the total number of cells counted, p is that fraction of the total made up of the particular cell type in question, and q is that fraction of the total composed of all the remaining cells. Barnett checked the application of this theorem to the differential count experimentally by a series of counts of 100 cells each, made on cover slip preparations from the same sample of venous blood, and found reasonably close agreement between the observed and predicted cell values. He has constructed a chart showing maximum errors due only to chance in differential counts based on the enumeration of 100, 200 and 400 cells. The maximum error he defined as three times the standard deviation. Thus if 100 cells are counted and 50 per cent are neutrophils the standard deviation is 5 per cent and the maximum error is 15 per cent. When the total number of cells is 400 instead of 100 the respective values are halved. Barnett concludes that at least 400 cells should be counted in order to obtain reliable results in the differential count.

The validity of the above theorem, as applied to differential counts, was also tested by Plum (1936), who counted on a number of slide films from 50 to 100 sets of cells, each set being composed of from 50 to 100 members. He examined

consecutive adjacent longitudinal rows, starting at the margin and "proximal" end of the film. The results showed that, in these experiments, the individual counts were distributed about the mean in the manner of the normal curve.

The normal frequency or Gaussian curve is not strictly representative of the distribution of most cell values in the differential count, even though the "sampling error" is assumed to be the only source of variation, according to Goldner and Mann (1938). However, by a mathematical analysis, they demonstrated that the error involved in the application of the normal curve to all counts is negligible. Based on this approximation they have constructed "confidence curves" for differential counts of 200 leukocytes employing a "probability" of 95 per cent ("Fiducial Coefficient" of 0.95). The validity of the curves was tested by serial observations of a single sample of blood. For leukocyte percentages ranging between 30 and 70 the calculated "maximum" variation is approximately $\pm$ 6 per cent.

The several sources of possible error in the performance of the differential leukocyte count have been considered by MacGregor, Richards and Loh (1940) with particular reference to variations dependent upon technique of making the film and method of selection of areas for counting. On all slide preparations lack of uniformity of distribution of cell types was found to be the rule. Variations, in some cases exceeding 20 per cent, were attributable to this factor. A general relationship was observed between the wave distribution pattern of one type of cell and that of any other type. In order to determine this relationship consecutive areas throughout slide films were counted and the values obtained subjected to Fourier analysis. From the data secured in this manner graphs were constructed. These graphs enable correlations to be made between the count determined by examination of all cells in the film and the results obtained by 1, "edge" count, along the two longitudinal margins of the film for a width of one microscopic field; 2, "battlement" count, three horizontal edge fields followed by two fields toward the center, then two horizontal, and then two in a vertical direction back to the edge; 3, "cross-sectional" count, back and forth across the body of the film from one edge to the other until at least 300 cells have been counted. It therefore becomes possible, according to the authors, to compare results obtained by different methods of counting by the application of correction factors varying with the type of cell and its observed percentage value. The accuracy of such "corrections" is dependent upon a standardized technique of film preparation and is lessened in cases of total leukocyte counts differing widely from the normal. MacGregor and his associates found that closest approximation to the values obtained by a count of the entire film was provided by the battlement method utilizing both edges of the film. Greatest corrections were necessary in the case of counts made by the cross-sectional method, with the straight edge method occupying an intermediate position. Counts performed on cover slip films correspond more closely to the straight edge count than to either of the other two methods of counting on slide preparations.

The percentage of lymphocytes in the case of a healthy individual was found by Boveri (1939) to average about 10 per cent higher when determined on slide

films by the cross count technique described above, than when enumerated in cover slip preparations or in a Buerker counting chamber. The counting chamber is assumed by the author to give most nearly perfect random distribution. On the basis of removal of four-tenths of the granulocytes and monocytes from the countable portions of a slide film the author has constructed a curve showing the corrections that should be made in order to obtain the true lymphocyte percentages from values obtained by counting slide preparations.

The four-field meander method, counting from one side of the film to the other and back, and differential enumeration of cells in a counting chamber were compared by Klotz (1939) for constancy of results when single samples of blood were used. The slide films were made in a uniform manner, standardizing the size of the drop, the angle of the spreading slide and the pressure exerted when making the spread. Random cross counting gave results considered to be more accurate than either the four-field or hemacytometer chamber methods.

From the rather widely differing conclusions of the several authors whose work has been described above, it is apparent that no agreement has been reached regarding either the preferred method of differential counting on slide preparations, or the degree to which distribution of cells approaches the wholly accidental on either slide or cover slip films. However, it has been the experience of most observers who have compared the slide and cover slip methods, that slide films show a distinct predominance of neutrophils and monocytes at the edges and tail of the preparation, and that even with a well standardized technique, this inequality of distribution varies with the concentration of the red cells and the number of leukocytes in the blood; whereas, in cover slip films, utilizing only the weight of the upper slip to effect the spread, the distribution of white cells is more nearly uniform. It is obvious that the method employed in selection of areas for differential counting becomes less important as the distribution of cells approaches the "accidental."

DAILY AND HOURLY VARIATIONS. It has long been known that fluctuations in the leukocyte count may occur throughout the day, but originally these were attributed to exercise or digestive activity. Later it was recognized that they arise independently of these possible excitants. In 1925 Sabin, Cunningham, Doan and Kindwall reported that a characteristic hourly rhythm of the total white blood cell count occurred in man and rabbits, and that this accounted for a variation in a proportion of 1:2. Furthermore, they found that the white blood cell count was increased in the afternoon, independent of food intake, and that the entire increase was due to changes in the number of neutrophils. They also concluded that the lymphocytes have a shorter rhythm than the neutrophils, and that their total number varies in the proportion of 1:3. The suggestion that oscillations characteristically occur at hourly intervals has not been confirmed. Casey, (1940), after a most extensive study, concluded that his studies on rabbits did not reveal "a statistically significant variation in the hourly mean." Kennon, Shipp and Hetherington (1937) in an investigation of the leukocyte count in young men likewise failed to discover "proof of rhythmicity occurring at regular intervals of short duration, either for the total number of

leukocytes or the total numbers of any one type of cell." It is true, however, that variations of considerable magnitude in the white blood cell count, due to the changes in the numbers of granulocytes, do occur in the blood of rabbits and man. Furthermore, these alterations are not related to food intake or exercise. According to Garrey and Bryan (1936) if one substitutes the phrase *irregular and sometimes pronounced oscillations* for *rhythms*, the apparent disagreements in the literature are of minor importance. These observers conclude that when leukocyte counts are done in the morning, under basal conditions, a great majority of the white blood cell counts will fall within the range of 5,000 to 7,000 cells per cubic millimeter. According to them, it is firmly established that the minimum count is observed in the morning, under conditions of rest, and that it varies least under these conditions. Furthermore, they state that after an hour of recumbent rest in the afternoon, 90 per cent of all leukocyte counts fall within the limits of the morning basal counts.

In the study of Kennon, Shipp and Hetherington (1937) the white blood cell count in six young men was determined at intervals of 15 minutes throughout the day for 5 to 7 hours, on two successive days. They observed that the total number of leukocytes varied from count to count, due to changes in the granulocytes, but no evidence of a rhythm could be determined; the undulations in the curve had a tendency to adhere to the same pattern in a given subject on successive days; after a rest of one-half to one hour there was a fall in the leukocyte count to the basal range; there was no significant alteration in the total white blood cell count when an erect posture was assumed; the apparent spontaneous variations in the white blood cell count throughout the day were very slight, usually the difference being between 100 and 400 cells between the highest and lowest counts, but in one instance the maximum variation was 900, and in another, 1800 cells; while there was no steady consistent rise in the white blood cell count in the afternoon, in all instances there was a definite post meridian peak between twelve noon and 3:00 p.m.; and although the subjects had breakfast or a light lunch during the days of the study, there was no evidence of a digestive leukocytosis.

Casey (1940) undertook a comprehensive statistical evaluation of a large number of white blood cell counts in normal rabbits in order to determine if a recognizable diurnal cycle in the leukocyte series actually existed in this species. The counts had been made not oftener than three times a week in a given animal, and usually only once a week. In all, 597 estimations on 190 of the 204 rabbits were made between 9:00 a.m. and 12:00 noon, and 366 determinations of 136 of the 204 rabbits between 12:00 noon and 5:00 p.m. It was concluded that no single individual hourly mean differed significantly from any other; what variation was present, they considered, could be accounted for on the basis of random sampling. The white blood cell count averaged 7,891 per cu. mm. for the morning, and 7,914 per cu. mm. for the afternoon period, a difference of 23± white blood cells which is not significant. This investigator also concluded that there was no evidence that a digestive leukocytosis occurs in the rabbit between the hours of 9:00 a.m. and 5:00 p.m., as no statistically significant change was ob-

served in the group permitted free access to food and water. It is recognized by Casey that previous observers have reported that the lowest white blood cell counts in man occur early in the morning and the highest in the afternoon, despite control of the food intake, muscular effort and emotional stress. It is also pointed out that repeated painful punctures cause a successive rise in the leukocyte count (Garrey, 1929). Furthermore, Casey is critical of most of the studies of the variations and rhythms in the white blood cells, as he considers that they are based on an inadequate number of estimations, and no statistical consideration of observational variation seems to have been undertaken. His study, he believes, is significant and conclusive for a number of reasons, among them being: only a single examination was made on an animal on any one day; the material was analyzed by accepted biometric procedures; it represents by far the most extensive study of the sort, which has been made. Certainly, his results seem, in general, to contradict the belief that there is a uniform diurnal rise in the leukocyte count in rabbits. It cannot be said, however, from the material presented, that individual rabbits do not show a diurnal rhythm, or an increase in the leukocyte count in the afternoon hours.

As a prelude to a study of the variation in the leukocytes in rabbits following the injections of various substances, Reifenstein, Ferguson and Weiskotten (1941) have made a most careful study of the spontaneous variations at hourly intervals in the total leukocyte count, the differential formula, and the maturity of neutrophils in normal rabbits. Throughout the experiments there was careful control of the health and handling of the animals, the environment, and the diet. Adequate precautions were taken to ensure the accurate estimation of leukocytes as 16 unit areas of the chamber were counted which meant that 800 cells were actually enumerated, when the total leukocytes numbered 10,000 per cubic millimeter. Eight leukocyte estimations were made at hourly intervals on the same day between approximately 9:00 a.m. and 4:00 p.m. Three blood films were made at the time of each count, and 300 cells were enumerated in order to calculate the differential formula. A group of fourteen adult New Zealand white and mixed rabbits of both sexes, weighing approximately 2.5 to 3.2 kgm. each, were studied during the period from May 24, 1939, to January 23, 1940.

The results show that there is considerable spontaneous variation in the leukocyte counts of each rabbit. This is apparent when the average of all eight counts on each day for each rabbit are compared. The average for the series of the fourteen animals was 9,449 per cubic millimeter with maximum average variation from 6,414 to 15,014 per cubic millimeter. Repeated studies on the same rabbits at approximately the same hours of the day, on different days, show that each animal seems to have its own characteristic leukocyte picture and variations appear to be decreased in extent under such conditions. The observers advise that when hourly data dealing with the leukocytes are determined for experimental purposes, it appears wise to employ the same animal after its individual leukocyte behavior has been determined, and then utilize the same period of the day for counting. When average hourly estimations for all of the fourteen animals are considered, the total leukocyte values are fairly constant;

the average number of neutrophils increase and the average number of lymphocytes decrease during the day. For example, the average hourly counts per cubic millimeter for the fourteen animals, from approximately 9:00 a.m. to 4:00 p.m. were as follows: 9,600, 9,221, 9,621, 9,914, 9,300, 9,800. On the other hand, the total leukocyte count in the same rabbits may vary from 7,000 per cubic millimeter at 9:00 a.m. to 14,900 at 3:00 p.m. This is not true of all of the animals for in some the count may have a remarkable constancy. A comparison of the average morning neutrophil counts with those in the afternoon shows that almost invariably there is an increase in these cells during the latter part of the day. This varies from approximately 200 to 2,000 cells per cubic millimeter. Occasionally there is a decrease. With the tendency to an increase in the neutrophils during the afternoon, there is usually a decrease of the lymphocytes of approximately 200 to 3,500 cells per cubic millimeter. Here again there may be an occasional increase. The authors conclude their discussion with a very appropriate and practical warning, as follows: "These results seem to indicate that when the hourly leukocyte picture of the rabbit is studied under different experimental conditions, it would seem advisable to determine the general normal leukocyte trends of each animal and then maintain the same period of the day for counting at hourly intervals under experimental conditions, as well as establish similar control of other possible sources of error in the determinations."

An additional study by Reifenstein and Hilfinger (1942) merits careful consideration for they investigated in rabbits for the first time the variation in the maturity of polymorphonuclear neutrophilic leukocytes from hour to hour during the day. Total and differential white blood cell counts were made at hourly intervals at the same time of the day for six hours on twenty normal adult animals. The cells were classified according to the following grouping:

Group I. Cells with the nuclear material in one mass, round, oval, or indented not more than one-half through its width.

Group II. Cells with the nuclear material not divided into segments but may be lobed, spiral, looped, rosette shaped or variously irregular.

Group III. Cells with nuclear material showing segmentation into two parts which are either entirely separate (as viewed in the film), or connected with a narrow filament.

Group IV. Cells with the nuclear material showing segmentation (as in group III) into more than two parts.

According to this arrangement, the first and second groups comprise the division known as the non-filamented, and groups III and IV the filamented neutrophils.

These results are expressed in the weighted mean which has the advantage of indicating the maturity of 100 neutrophils by a single figure. It is computed as follows: The number of cells in each of the four groups is multiplied by the number of the group (I, II, III or IV), all results are added and the sum is divided by the total number of cells counted. According to them, the weighted mean is an exceedingly sensitive index of nuclear classification as a difference of one point

in the second decimal place means that one neutrophil has been moved to the group either below or above its original assignment.

A consideration of the results shows that each animal on its particular day of counting appeared to have a rather characteristic weighted mean. The only other change was that some seemed to be "more to the left" than others. There did not seem to be a tendency for the mean to shift to the left or right during successive hourly counts. In other words, there was a strong tendency to maintain the initial average neutrophilic maturity during the six hour period of counting.

The percentage of the non-filamented neutrophils (groups I and II) varied from 11 to 48, with a mean of 28.5, median of 29 and a mode of 29. Only two group I-neutrophils were observed in classifying approximately 14,000 neutrophils. One table is presented showing the neutrophilic maturity counts on the same animal at approximately the same time of day (9:45 a.m.) on different days during a 23 months period. These data emphasized that while the level of the neutrophilic weighted mean in a rabbit may vary slightly over a long period of time, the relative constancy of the mean, once it has been established for any particular daily period of study, is maintained during this interval. In other words, when a general trend in the neutrophils has been determined for each animal, there is every reason to expect that there will be very little shift to the "left" or "right" during the succeeding six hours. These results would indicate that the ratio of the appearance of younger neutrophils in the blood to the disappearance of the older neutrophils from the blood is balanced or constant. The scarcity of group I cells suggests that rarely are cells of the neutrophilic series discharged into the blood until they reach a stage which is more mature than the metamyelocyte stage. Their studies would suggest also that any variation in the neutrophils which does occur at short intervals is not due to an increase in the more immature cells of this series. It is likely, therefore, that such variations are on the basis of a redistribution of the cells of the body.

Medlar (1929, 1936), after an exacting study on normal persons and on patients with tuberculosis, reached the following conclusions concerning the fluctuations in the white blood cell count. In his opinion neither the total leukocyte count nor the percentage of the different types of white corpuscles has an absolute level. A variation in the total of 50 per cent and in the differential count of 10 per cent may occur in normal persons when counts are made at five to ten minute intervals, over a period of an hour or two. Such variations may occur at irregular and unpredictable intervals. He cautions that a significant fluctuation must, therefore, be more than 50 per cent of the total and 8 per cent of the differential picture. Furthermore, to be pertinent, it is necessary that the changes occur uniformly in subsequent observations. He noted no significant effect of rest and random activity on the total and differential count. This led him to conclude that distinctive "rest" and "activity" levels of the leukocyte count do not exist if the activity is limited to mild exercise. He is also of the opinion that "complete relaxation, such as a night's sleep or two hours in bed, appears to bring about a more irregular distribution of the leukocytes, both in

the total and in the differential count, than is present during a state of normal activity."

THE EFFECT OF ACTIVITY ON THE LEUKOCYTE COUNT. It appears to be clearly established that the leukocytes increase in numbers in the circulating blood following muscular exertion. This is a constant finding and, furthermore, the leukocytosis is, to a certain extent, proportional to the duration and severity of the muscular contractions. Reviews of the literature bearing on this subject have been published by Grawitz (1911) and by Garrey and Bryan (1935). Exercise of any type increases the leukocyte count, even "random activity" causing a moderate rise in the count. It has been established that the leukocyte level is lowest during periods of minimal activity (Bryan, Chastain and Garrey, 1935) and that the correct basal count is the one made in the early morning under conditions of rest (Naegeli, 1931). Following the initial observations of Schultz (1893) it was shown that Marathon runners at the termination of a race uniformly had a leukocytosis which varied between approximately 14,000 and 27,000 cells per cubic millimeter, and a percentage of polymorphonuclear leukocytes which ranged from 80 to 90 per cent of all the white blood cells. It is generally believed that the increase is due to a redistribution of the white blood cells in the vascular system rather than to a formation of new leukocytes. This is indicated by the rapidity with which the increase may occur and by the fact that only a small percentage of the cells are of the younger types. It has been recorded immediately following a quarter mile race, lasting less than one minute, that there is a leukocytosis of 35,000 per cubic millimeter. Isaacs and Gordon (1924) report that at the termination of a Marathon race the polymorphonuclear leukocyte count percentage may be as high as 94.7. That this increase is attributable to a redistribution of cells in the vascular system is shown by the fact that in all cases examined by them the polymorphonuclear neutrophil leukocytes appeared to be of the mature, adult type, with clear-cut nuclear lobulation. It is generally believed that the changes in the circulating blood following exercise are due to circulatory shifts with the liberation of sequestered leukocytes from unused capillaries throughout the body. These reservoirs, in which the polymorphonuclear cells are stored, may be in various organs and tissues of the body such as the spleen, liver, lungs, glands of internal secretion, bone marrow, and muscles.

That the degree of leukocytosis is due to the intensity and the duration of the exertion is shown conclusively by the observations of Edwards and Wood (1932) who noted an increase of nearly 300 per cent in the white blood cell counts of football players although the average duration of actual play in a sixty-minute game is only eight minutes. This degree of leukocytosis is surpassed only by that observed in Marathon runners. Furthermore, it was found that the degree of leukocytosis was directly proportional to the extent of their participation in the game, as shown by the following figures: after playing one-quarter, the average count was 12,000 per cubic millimeter; one half, 15,500; three-quarters, 18,000; and the entire game 23,000.

It is claimed by some that the emotions of fear, rage and apprehension may

raise the count to activity level (Garrey, 1929) but Edwards and Wood (1932) consider that excitement alone is without effect upon the leukocyte count. This they demonstrated by determining the leukocyte count on spectators at games, football players immediately before games, and track athletes just prior to a race. Leslie and Zwemer (1935) failed to obtain an increased leukocyte count in cats with excitement alone and therefore concluded that they were unable to show any increase in the white blood cell count from emotional stimulus in the absence of activity. For a further discussion of this aspect of the subject see under "Emotion."

It is claimed that lactic acid of the blood, blood sugar, blood pressure, body temperature and capillary dilatation can be ruled out as separate causes related to leukocytosis in exercise (Edwards and Wood, 1932). The leukocytosis of exercise seems to be proportional to the extent of the alterations in the circulatory rate of the body. As this is accelerated, the sequestered cells are swept into the circulation causing a physiological leukocytosis. Furthermore, they remain there for a considerable period of time before, as it is reasonable to assume, they gradually settle out again in the various capillaries of the body when the circulation becomes slowed. Leslie and Zwemer (1935) believe that the main factor in the prompt production of leukocytosis following exertion is the dilatation of collapsed capillaries in the muscles of the body. They state that during rest there are vast numbers of leukocytes adherent to inactive vessel walls. These become detached when the capillaries open during exercise, are swept into the circulation, and account for the rise in leukocytes. The possibility that the spleen may harbor a reserve store of leukocytes which enter the general circulation when this organ contracts following adrenalin injections, thereby causing a transient leukocytosis, has led some to emphasize that this viscus may play an important rôle in the production of physiological elevation of the white cell count. This is undoubtedly true but it has been demonstrated that the injection of adrenalin will cause such a leukocytosis in persons following splenectomy (Lucia, Leonard and Falconcer, 1937). A more likely explanation of the increase in number of leukocytes is that the adrenalin increases the rate of blood flow and thereby redistributes the formed elements of the blood. Thus it is not difficult to understand why there is a leukocytosis in exercise of any type, or, in fact, in any condition in which the circulatory rate is increased. This may occur in random activity, in the labor of a pregnant woman, in athletic contests, following the injection of adrenalin, in excitement, and following convulsive seizures.

It should be pointed out that in some persons the leukocytosis is due to an increase in lymphocytes instead of polymorphonuclear cells. For example (Garrey and Bryan, 1935), a high grade of leukocytosis may show a preponderance of lymphocytes. It is also reported (Jokl, 1931) that the blood picture undergoes three changes following exercise: first, an increase in the percentage of lymphocytes following strenuous exercise such as a race of 2,000 meters; second, a preponderance of polymorphonuclear leukocytes after a race of long duration (10 to 25 kilometers) and, third, an "intoxication picture" after a

Marathon race when the percentages of polymorphonuclear cells and lymphocytes are normal. The first two types of change have been observed by others but not the third, as previous work has shown that the picture in the blood following Marathon races is that of a polymorphonuclear leukocytosis. That a lymphocytosis may follow a bout of short, intensive exercise is to be expected in some instances, because the exertion has been shown to increase the flow of lymph (Rous, 1908). The original view of Erhlich was that a lymphocytosis could result from an increase in the rate of production of the lymphocytes in lymphoid tissue, and also from a flushing out of the lymph cells through an increase in the lymph flow. The classical observations of Rous confirm the view that the lymphocytes, too, like the polymorphonuclear cells, are sequestered but in the lymphatic rather than the circulatory system, and that following exercise they are redistributed to the circulating blood thereby causing an increase in the percentage of these cells. The experiments of Rous prove the existence of a large reservoir of lymphocytes, stored in the lymphatic system, which is quickly yielded to the blood following exercise.

Thus in any person there are at least two ever present variables, which are responsible for the changes in the white blood cell content of the circulating blood. One of these is the reserve supply of polymorphonuclear leukocytes which is sequestered in the inactive capillaries of the body. The other is the segregation of lymphocytes in the lymphatic system. Cells of both types are returned to the circulating blood following muscular activity but in one person the lymphocytes may be swept into the circulation first, while in another it may be the polymorphonuclear cells. In general it may be said that the lymphocytes more commonly respond to short severe exercise whereas in prolonged exertion the leukocytosis is of the polymorphonuclear type.

In the course of a comprehensive investigation of the problem of fatigue and hours of service of drivers of commercial vehicles operating in interstate commerce, Donahue of the Public Health Service (1941) observed the influence of driving upon the leukocyte picture of truck drivers.

Preliminary studies indicated that the leukocyte count might be utilized as an index of the fatigue status of commercial truck operators. It was found that the average leukocyte count of 737 drivers was 9,811 per cubic millimeter, with a range varying from 4,900 to 19,200 per cubic millimeter. These figures were recognized as higher than those usually reported for the general population. There was a statistically significant difference between the counts in different cities, for in Baltimore, Chicago and Nashville the average counts were 9,010, 9,713 and 10,712 per cubic millimeter, respectively. This variation may have been due to a variation in the time of working hours, or different economic conditions. No age trends were observed in the total leukocyte count. The mean values of the leukocyte counts of all drivers showed a consistent tendency toward an increase with hours of driving since major sleep. The rise was found to be relatively slight as compared with subjects undergoing severe muscular exercise. It was concluded that "these results are suggestive of a chronic effect of the regime of the truck driver on the leukocyte level."

The effect of either the increased muscular activity associated with a pronounced tachycardia, or of the accelerated circulatory rate, or of both of these factors, is well demonstrated by the observations of Levine and Golden (1922) on patients with greatly increased heart rates. Eleven patients with paroxysmal rapid heart action were studied in whom the rate varied between 130 and 250 per minute for two hours to over twelve days. In six of these the leukocytosis ranged between 13,000 and 22,000 per cubic millimeter. The authors considered that the blood findings were due to the cardiac upset, as a return to normal occurred promptly when the rate was lowered.

Bookman and Fraad (1935) studied the effect of prolonged training on the neutrophil count. They made weekly observations on 14 members of a college football team during the period of approximately eleven weeks of almost daily strenuous exercise. No significant variations were found in the percentage of neutrophils and hence it was concluded that prolonged exercise and training do not have an appreciable effect on the polymorphonuclear counts. They emphasize that this is rather remarkable as the total white blood cell count during energetic practice and actual contests must have fluctuated violently.

VARIATIONS WITH AGE. According to Poncher (1943) the average leukocyte count shortly after birth is 25,000 cells per cubic millimeter. The level falls rather precipitately to about 14,000 at the tenth day of life, with a subsequent very gradual decline throughout infancy and childhood. Poncher's conclusions appear to be based, in part, on the observations of Kato (1935). Washburn (1935) reported a total of 73 leukocyte counts on six babies performed during the first ten days of life. Wide variations were found both between different infants, and between counts on the same babies; the average of all the counts was 15,208 per cubic millimeter. For seven infants between two and twenty-six weeks of age a total of 535 leukocyte counts varied between 5,000 and 24,000, with 80 per cent of the counts within the range of 8,000 to 16,500. Washburn observed no tendency to orderly rhythms in the fluctuations of the individual infants' counts, nor was any correlation noted between the counts and activity or feeding. Kato (1935) analyzed 1,081 total and differential counts performed on 1,037 individuals between birth and fifteen years of age. At the end of the first day of life he found an average leukocyte count of 22,000 per cubic millimeter with a pronounced drop at the third or fourth day to an average of 8,750. Thereafter a slight temporary rise occurred during the first three months of life with an average maximum level of 10,000 to 11,000. A gradual fall was then observed, persisting throughout the remaining years of infancy and childhood.

The values usually given for the leukocytes in normal infants and children are criticized by Osgood as being derived from studies on subjects not truly healthy. With a number of collaborators (1939, 1941) he has reported total, differential and absolute leukocyte counts for new-born infants and for age groups above 4 years. During the first day of life the leukocyte count averaged 15,000 with maximum values of about 24,000. A fall to an average of 10,000 was observed on the second day with a further decline to 7,000 on the third, fourth, fifth and sixth days. The count then increased to about 8,000 where

it persisted throughout the remainder of the ten day period of observation upon which the study was based. In a study of 86 children four to seven years old, Osgood and his associates found an average leukocyte count of 10,400 with a 95 per cent range of 5,500 to 15,500. Scatter diagrams indicated that, within these ages, the values for white blood cells are constant. At eight years the total leukocyte count declines, according to these authors, to an average level of 8,400, range 4,000 to 13,000, and these values persist through adolescence. A further decrease occurs after the age of eighteen, when the adult values were established as mean total leukocyte count 7,400, range 4,000 to 11,000. The authors observed no differences between the sexes, either in total or differential leukocyte counts, in any of the age groups studied.

The white blood cell values at four periods in the first year of life were studied by Magnusson (1938). He provides an extensive bibliography of articles published prior to 1936. His own observations made on carefully selected subjects are shown in table 2.

TABLE 2

AGE	NUMBER OF COUNTS	LEUKOCYTES (THOUSANDS PER CU. MM.)	
		Mean	Standard deviation
weeks			
3rd	16	14.18 ± 0.77	3.08 ± 0.54
11th	20	10.79 ± 0.62	2.77 ± 0.44
23rd–25th	20	13.41 ± 0.62	2.77 ± 0.44
49th–52nd	20	12.82 ± 0.72	3.23 ± 0.51

In a study of approximately 300 healthy infants, aged 4 to 6 months, Bethell (1943) found an average leukocyte count of 11,279, standard deviation of 1,012 cells per cubic millimeter. At 10 to 14 months the mean count was 12,358, standard deviation 3,886. In the older age group the flatness of the frequency distribution curve, with some skewness toward the higher values, may be due to the inadvertent inclusion of children with subclinical pathologic processes. If so, it illustrates the difficulty involved in the selection of normal subjects for the determination of leukocyte counts applying to young children. Relatively mild infectious processes may produce either leukocytosis or leukopenia, depending upon the responsible etiologic agent, and such changes tend to persist for long periods after the disappearance of clinical evidences of disease.

At birth the neutrophils are the predominant type of leukocyte in the circulating blood. Their relative and absolute numbers fall rapidly, and during the second week are exceeded by the lymphocytes. After the first year the lymphocytes gradually decline but remain the most numerous cells until about the fourth year when they are surpassed by the increasing percentage of neutrophils. According to Poncher (1943) the number of granulocytic and lymphocytic types approximate each other at the tenth day of life and again at the fourth year. Eosinophils and basophils remain at a relatively uniform level throughout infancy and childhood, and their numbers are about equal to the adult values.

TABLE 3

*Mean leukocyte values at different ages**

AGE	AUTHOR	TOTAL W.B.C. 000/CU. MM.	NEUTROPHILS	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONOCYTES
			per cent	*per cent*	*per cent*		
Newborn	Poncher† 1943	25,000	60.0	2.5	0.5	22.5	10.0
	Chuinard‡ 1941	15,000	60.0	3.0	0.5	30.0	5.0
10th day	Poncher†	14,000	40.0	2.5	0.5	42.5	7.0
	Chuinard‡	8,000	43.0	3.0	0.5	45.0	5.0
4th–6th month	Poncher†	12,000	32.0	2.5	0.5	52.5	4.0
	Magnusson 1938	13,410	24.18	3.09	0.21	64.16	7.32
	Bethell 1943	11,279	25.0	1.80	0.15	69.33	3.61
10–12th month	Poncher†	9,000	32.0	2.5	0.5	57.0	4.0
	Magnusson	12,820	29.52	2.54	0.25	60.63	6.41
	Bethell	12,358	28.62	2.46	0.14	65.54	3.65
	Suzuki§ 1937	11,000	27.5	2.0	0.6	62.5	5.0
4th year	Poncher†	9,000	42.0	2.5	0.5	45.0	4.0
	Osgood‡ 1939	10,400	41.0	3.0	0.5	48.0	3.0
	Suzuki§	8,500	45.0	2.5	0.6	45.0	5.5
7th year	Poncher†	8,000	50.0	2.5	0.5	33.0	4.0
	Osgood‡	10,400	41.0	2.0	0.5	48.0	3.0
	Suzuki§	8,000	50.0	2.5	0.6	40.0	5.5
12th year	Poncher†	8,000	52.0	2.5	0.5	28.0	4.0
	Osgood‡	8,400	41.0	2.0	0.5	48.0	3.0
	Suzuki§	7,000	60.0	3.0	0.7	30.0	6.0
15th year	Osgood‡	8,400	51.0	2.0	0.5	42.0	4.0
	Suzuki§	6,500	65.0	3.0	0.7	23.0	6.0
Adult	Osgood‡	7,400	55.0	2.0	0.5	38.0	4.0
	Suzuki§	6,000	67.0	3.0	0.7	23.0	6.0

* It has not been possible to establish statistically comparable ranges for the data included in this table. It is designed to indicate the trend of change throughout infancy and childhood. Apparently healthy individuals, especially in infancy, may give values differing widely from the means. Where the summation of percentages falls appreciably short of 100, the discrepancy is due to omission of unidentified and disintegrated cells. The numbers of observations from which these figures were derived are comparatively large with the exception of those of Magnusson. This author, however, chose his subjects with especial care and subjected his data to detailed statistical analysis.

† Approximate values calculated from the authors' ranges.

‡ Smoothed means, with some values adapted from the authors' classification to fit the present table.

§ Data largely compiled from the reports of many authors, chiefly in the Japanese and German literature.

The mean values for the total and differential count throughout infancy, childhood, adolescence and adulthood, as observed by several recent authors, are given in table 3.

PREGNANCY, LABOR AND THE PUERPERIUM. Most observers report an elevated leukocyte count during pregnancy, the increase being due to greater numbers of neutrophils, with a higher percentage of younger forms than is found in non-pregnant women. Carey and Litzenberg (1936) report 977 leukocyte counts obtained during the course of 134 pregnancies. The median value of these counts was between 10,000 and 11,000 per cubic millimeter, with no consistent alterations throughout the duration of gestation. Fifty per cent of the counts fell in the range of 8,700 to 12,500. No differences in leukocyte counts between primiparae and multiparae were observed. Hematologic data

TABLE 4

Leukocyte values in pregnancy, the puerperium and at approximately one year after delivery (Bethell, Hartsuff and Farrell, 1943)

MONTH OF PREGNANCY	LEUKOCYTES (THOUSANDS/CU.MM.)			DIFFERENTIAL COUNTS (MEAN PERCENTAGES)					
	No. of subjects	Mean	S.D.	No. of subjects	Neut.	Eos.	Bas.	Lymph.	Mono.
2nd	11	10.182	2.886	10	58.00	2.90	0.00	34.00	4.90
3rd	46	10.022	2.445	41	66.71	1.67	0.17	27.85	3.93
4th	81	10.420	2.154	79	67.66	2.17	0.11	27.51	3.35
5th	125	10.888	3.112	114	67.72	1.68	0.15	26.96	4.11
6th	154	10.539	2.543	147	69.56	1.49	0.12	25.21	3.98
7th	200	10.875	2.435	188	69.68	1.39	0.15	25.30	4.41
8th	226	10.518	2.975	190	69.47	1.58	0.12	25.11	4.47
9th	174	10.339	2.515	149	68.96	1.52	0.09	25.32	4.46
Postpartum									
5th–9th week	343	8.192	1.858	334	51.29	2.84	0.31	41.28	4.26
10th–14th month	111	8.072	.610	107	54.63	2.54	0.26	39.52	3.83

obtained on 36 women at different periods of gestation were reported by Olivella, Chediak and Ballestero (1938). They found average values for the total leukocyte count of 9,250 cells per cubic millimeter; neutrophils, 68 per cent; eosinophils, 2 per cent; lymphocytes, 23 per cent; monocytes, 4.5 per cent.

Blood studies were carried out by Bethell, Hartsuff and Farrell (1943) on healthy women in various months of their pregnancies, at approximately six weeks postpartum, and at about one year after delivery. In all, 1471 total leukocyte counts and 1359 differential counts were obtained. Although statistical analysis of these data has not been completed, it is possible to observe the general nature of the leukocyte changes in pregnancy and the puerperium as evidenced by the figures in table 4. The total leukocyte count remains constantly elevated throughout pregnancy with relatively minor changes from month to month. There is a persistent neutrophilia which increases through the seventh month of gestation and then appears to undergo a slight decrease.

Lymphocyte, eosinophil and basophil percentages are lower than in non-pregnant healthy adults, but the absolute values for these cells are but little affected during gestation. The percentage of monocytes is approximately the same in pregnant and in non-pregnant persons, but their absolute number is increased in the former group. Throughout pregnancy the range of leukocyte values is very great as indicated by the high figures for the standard deviations. The total count declines and becomes more uniform at the 5th to 9th week postpartum, the decrease being at the expense of the neutrophils. The data obtained at approximately one year after delivery may be considered as representing the normal non-pregnant leukocyte values for the subjects of this investigation.

Changes in the leukocyte count during labor have been studied by Gibson (1937) who observed a slight rise in total cell values at the beginning of parturition and a much greater increase immediately after its termination. Counts in the range of 10,000 to 39,800 were found during the first six hours after delivery in 38 normal women. In primiparae the leukocyte values tended to be higher and the leukocytosis to persist for a longer time than in multiparae. The leukocytosis of parturition was made the subject of an investigation by Boyd, Blenkinsop and Mylks (1937), with particular reference to the lipid composition of the white blood cells. In 14 patients the average leukocyte count in early labor was 7,100 cells per cubic millimeter, the lowest being 4,300 and the highest 10,000. At the end of labor, but before anesthesia, the average was 8,000, with a range of 5,700 to 11,200. In every instance some increase in the count occurred, averaging 1,700 cells, a 24 per cent gain over the values early in labor. No changes either in the differential count or in the lipid composition of the cells between beginning and advanced labor were observed. Earlier publications on the leukocyte count in labor have been reviewed by Wolff (1941), who reports studies on fifty normal women observed from shortly after the onset of labor until the conclusion of hospitalization at the eighth day after delivery. The number was equally divided between primiparae and multiparae. Twenty-one of the subjects were also examined at some time during the last weeks of pregnancy when their leukocyte values averaged 8,054 cells per cubic millimeter, with a range of 5,750 to 12,200. In all of these women a rise averaging about 2,000 cells was noted after the onset of labor, as compared to the previous counts. As labor progressed the leukocyte values increased to maximum averages at delivery of 22,250 and 15,270 in the cases of primiparae and multiparae respectively. The third stage of normal labor was not associated with further changes in the counts. The leukocyte values gradually returned to a normal range by the seventh day of the puerperium. Wolff states "that the work of the contracting uterine muscle appears to stimulate the mobilizing of the polymorphonuclear leukocytes into the systemic circulation."

Emotion. Leukocyte alterations, both quantitative and qualitative, associated with affected states have been reported by many investigators. The subject is discussed extensively by Garrey and Bryan (1935). In rabbits, including splenectomized animals, Nice and Katz (1936) observed, consistently,

leukopenia occurring during excitement; whereas in cats the same stimulus was followed by leukocytosis. In another article Katz and Nice (1936) reported no change in the ratio of non-filamentous to filamentous neutrophils in the blood of rabbits during excitement. They conclude that the emotional leukopenia of rabbits appears to be due to the sequestration of neutrophils in the peripheral capillaries and tissues.

In humans with emotional disorders Milhorat, Small and Diethelm (1942) observed the frequent occurrence of leukocytosis. The subjects of their investigation comprised 200 psychiatric patients and no correlation was noted between the level of the white cell count and the specific psychiatric disease, although the degree of leukocytosis paralleled to a certain extent the intensity of the emotional reaction. Fear, agitation or anger characterized the emotional states, associated with leukocytosis. Improvement in the subjective reaction was followed by a return of the leukocyte count to normal levels. Although no consistent changes, either in the total or differential leukocyte values, were observed by Beccle (1935) in a study of 20 psychotic patients, he observed an increase in the percentage of cells containing "toxic" (basophilic with Romanowsky stain) and Sudanophile granulations. The extent of such granulations varied directly with the intensity of the mental disorder. Hematologic values, including total leukocyte and differential counts, were determined by Hill and Taylor (1938) in 21 subjects with pathologic anxiety states. The results were subjected to statistical analysis and showed no significant differences from the data afforded by a control group. However, in comment, it appears from the authors' protocol that their subjects were not in a state of severe emotional stress at the time of examination.

THE EFFECT OF DIETARY CHANGES AND STARVATION. A physiologic study by Forbes, Johnson and Consolazio (1941) of the blood of share croppers in Mississippi showed that a majority of the apparently healthy negroes who were observed had a leukopenia with related neutropenia, whereas white persons living under the same conditions had normal white blood cell counts. The average leukocyte count of 23 negroes was 4,050 cells per cubic millimeter, whereas the average count of 7 white persons living under the same conditions, and said to be partaking of similar diets, was 7,600 per cubic millimeter. Some of the percentages of neutrophils in the negroes were as low as 22 to 34, and the average for the entire group was 50 per cent, while the average percentage in the white persons was 60. Iron medication caused an increase of the neutrophils in the blood of 18 negroes from an average of 1500 to one of 2700 per cubic millimeter. The authors state that the cause of the leukopenia is not apparent. That a dietary deficiency might have been an important etiological factor is a possibility but the evidence in regard to this is not clear. It was difficult to obtain an accurate picture of the eating habits of the negroes but there did not appear to be a pronounced dietary deficiency. Nevertheless, it is admitted that "occasionally they might be reduced to corn, beans, potatoes and salt pork." That the leukopenia may possibly be associated with a food deficiency is supported by the experimental work of Day and his associates (1940). It was

observed by these investigators that a leukopenia as low as 700 cells per cubic millimeter occurred in monkeys when they were fed the Goldberger black-tongue-producing diet.

Benedict and his collaborators, as reported in an extensive study on human vitality and efficiency under prolonged restricted diet (1919), made certain observations on the blood in normal subjects who received such a diet for intervals varying from 22 to 37 days. The food intake was such that approximately a 10 per cent weight loss was attained. Under this regimen there was no significant change in the total white blood cell counts. It was considered that there was a disturbance of the proportion of the various types of white blood cells, as the average per cent of lymphocytes was high (36 per cent), and the average of the neutrophils was low (56 per cent). In New England, at that time, the average normal percentages for lymphocytes was considered to be 22, and of neutrophils 64.

Early observations on the effect of fasting in man and animals on the morphological constituents of the blood have been extensively reviewed by Ash and reported by Benedict in his remarkable study of the changes occurring during prolonged fasting (1915). Unfortunately many of these reports are obviously marred by faulty technic and must be rejected. Almost every type of numerical change in the leukocytes has been reported. The results of Ash, however, can be accepted as accurate. He made a complete blood study on a healthy male who lived for thirty-one days without food and drinking only distilled water. It was concluded that in an otherwise healthy person, with restriction of mental and physical activities, the blood can withstand complete abstinence from food for a period of at least 31 days, without displaying any essentially pathological changes. There were some significant alterations, however, in the leukocytes which were interpreted as physiological. Their number increased rather rapidly at the onset of the fast, reaching 12,400 per cubic millimeter on the third day. There was a fall to 8,400 per cubic millimeter on the fourth day, after which there was a variation of about 1,000 per cubic millimeter until the 16th day when the count approximated the preliminary level. Observations could be made only for three days after the termination of the fast, and no important changes were noted during this interval. The fluctuations in the total leukocyte count were obviouxly due to changes in the number of the neutrophils. Ash had no explanation for the increase of neutrophils during the early days of the fast other than, as he says, a rather unscientific one, namely, that the neutrophils are ever on the defense for the organism, and that these cells are most sensitive to changes in body conditions. The increase cannot be attributed to alterations in the water content of the blood, to an obscure bacterial infection, or to a stimulating effect of the perverted products of metabolism, for there was no evidence that these factors were of importance. The paucity of accurate observations which deal with the relationship between the food intake and changes in the leukocytes, makes any generalization concerning this subject unwarranted. From the studies carried out under the direction of Benedict it does not appear likely that starvation and undernutrition result

in any significant changes. The observation of a low white blood cell count in negroes, and the leukopenia seen in animals which are given a black-tongue-producing diet, suggest the possible relationship between some components of the diet and the level of the total white blood cell count. Obviously the subject demands further careful studies before conclusions can be formulated.

DIGESTION. Whether or not there occurs a leukocytosis attributable to digestion is still a controversial question. The very extensive literature on the subject is reviewed by Arneth and Ostersdorf (1923) and Garrey and Bryan (1935). Within recent years comparatively few contributions to the subject have been made, and it is the consensus of most observers that diurnal variations of the leukocyte count are not dependent upon digestive processes. Inada (1936) observed leukocytosis in rabbits after the intravenous injection of a five per cent solution of glucose (0.1 gram per kilo body weight). The change in the leukocyte count was attributed to redistribution of the cells and was of the same type observed after feeding a diet high in carbohydrate or protein. Injection of minute fat globules caused no change in the white blood cells. Inada infers from his results that digestion leukocytosis in rabbits is related to the absorption of glucose, but is independent of the absorption of fat.

THE NORMAL LEUKOCYTE PICTURE IN A HOT CLIMATE. Heat and intense solar radiation are factors common to the subtropical environment of Iraq in which the leukocyte picture in healthy British soldiers was studied by Kennedy and MacKay (1936). They made the following observations: *1*, there was a relative reduction in the percentage of neutrophils, the mean being 56.6 with a minimum of 35 and a maximum of 75.5 per cent; *2*, the monocytes were increased, the mean being 13.7, the minimum 4.0, and the maximum 29.5 per cent; *3*, and the polymorphonuclear index showed a shift in the direction of immaturity. In addition, occasional abnormal cells were observed such as myeloblasts, promyelocytes, myelocytes, lymphoblasts, Türk cells and normoblasts. The average blood counts were within the normal range, as follows: Red blood cells, 5,375,000 per cubic millimeter; white blood cells, 8,780 per cubic millimeter; hemoglobin, 96.3 per cent (Sahli). The authors conclude that the only environmental agency affecting all subjects was the climate and that heat and solar radiation are the common factors to which the blood changes might be attributed. Heat may produce a transient increase in the circulating leukocytes, but its long continued action is unknown. It has been recognized that ultraviolet radiation produces changes in the circulating blood (Russell and Russell, 1927; Kennedy and Flint, 1930; Kennedy and Thompson, 1927). Russell and Russell (1928) have reported that sunlight, ultraviolet radiation and heat all produce an increase in the lymphocytes, and also that eosinophils are increased by irradiation. These authors state that when soldiers are transferred from a temperate climate to the tropics, there is a considerable increase in the lymphocytes and erythrocytes, and the neutrophils are diminished.

EFFECT OF HYPERPYREXIA UPON LEUKOCYTE COUNT. Changes in the leukocyte count were observed by Bierman (1934) following hyperpyrexia induced by exposure of patient to radiations with a wave length of about 30 meters.

In most instances the rectal temperature reached 104 to 106°F. and was maintained at this level for 3 to 4 hours by surrounding the patient with a hood containing carbon filament lamps. Hourly observations of the leukocyte count were made on patients suffering from various diseases. The initial change was a reduction of between 25 and 30 per cent in the total number of leukocytes which occurred in the first or second hours of treatment. This was due to a diminution in the neutrophils. Following this there occurred constantly an elevation in leukocytes whose maximum, amounting to approximately 80 per cent of the initial figures, occurred about the sixth to the ninth hour. The magnitude of the leukocyte response depended upon the height and duration of the temperature rise, but the maximum leukocytosis occurred several hours after the temperature had returned to the normal level. The highest leukocyte count recorded was 22,600 per cubic millimeter. As the increase was due mainly to changes in the total number of neutrophils, and as the staff (non-filamented) neutrophils showed the greatest rise, usually being between 200 and 300 per cent, it was concluded that the increase was due to stimulation of the bone marrow. The reviewers would like to comment that although the evidence that stimulation of the bone marrow seems to be clear, nevertheless the accelerated circulation rate which accompanies hyperpyrexia must contribute something to the transient increase in the leukocyte count by causing a redistribution of white blood cells. It is stated (Bierman and Fishberg, 1934) that during hyperpyrexia the velocity of the blood flow may be increased more than 400 per cent.

THE EFFECT OF METEOROLOGICAL ALTERATIONS. Variations in the leukocytes in relation to meteorological alterations have been studied by Berg (1938). In a previous communication Petersen and he (1933) had observed that the average counts in winter were higher than those in summer, while the degree of fluctuation was greatest in the latter season. A continuation of the study had led the author to conclude that in normal persons, a period of heightened systolic and diastolic pressure is followed by an increase in neutrophils and eosinophils with the appearance of more immature forms, and an elevation of the total white cell count. It was also found that the level and the extent of the seasonal fluctuations are influenced by constitutional habitus. For example, a person with a pyknic habitus was reported as having the highest level and also showed the greatest degree of fluctuations, whereas the strongly sympathicotonic individual not only had the lowest level, but also the lowest degree of fluctuations. References are given to authors who have noted variations in levels and fluctuations in the counts with constitutional differences. Data are presented showing the correlation which is claimed to exist between variations in the level of the leukocyte count, the character of the fluctuations, meteorologic episodes, and the pathologic condition of five patients suffering, respectively, with aleukemic lymphadenosis, myelogeneous leukemia, pulmonary tuberculosis (two patients), and chronic lymphatic leukemia.

THE EFFECT OF ALTITUDE. In 1933 Stammers studied the white blood cell counts of persons living in Johannesburg, South Africa, which has an altitude of approximately 6,000 feet and receives 73 per cent of sun light. He reported

that the mean per cent of the polymorphonuclear cells was 54.2 and of the lymphocytes 39.7. These values are about 14 per cent lower than the average normal percentage for the neutrophils and approximately 14 per cent higher in the case of the lymphocytes, according to the standards then accepted. As persons residing at high altitudes are exposed to more ultraviolet radiation, which had been shown by Clark (1922) to stimulate a relative lymphocytosis, it was assumed that this was the explanation of the changes observed.

Peterson and Peterson (1934) reported their study on healthy young adults in the vicinity of Butte, Montana, at an elevation of 5755 feet, with a sunlight percentage of 57. Their conclusions agreed with those of previous observers, namely, that a relative lymphocytosis occurs at high altitudes, as the mean lymphocyte percentage was 36.2, and that of the neutrophils 54.36. These findings are in accord with those of Ruppanner (1920) who studied the blood of persons in the Swiss Alps, and with those of Hartman whose observations were made on members of the German Expedition to the Himalayas (1931).

Changes in leukocytes attributable to high altitude and increased solar radiation show, at the most, only slight deviations from generally accepted normal values, although the trend of such changes appears to be suggestive.

DISPLACEMENT, DISTRIBUTION OR PSEUDO-LEUKOCYTOSES. Vejlens (1938) in his comprehensive and scholarly review on the distribution of leukocytes in the vascular system, contributes pertinent information relative to increases in the leukocytes in both physiologic and pathologic conditions. His observations indicate that normally in the veins some of the white corpuscles are axial flowing, and others are marginal and adherent to the vessel wall. These latter cells are always neutrophils. He believes that an increase or decrease in the number of marginal leukocytes in the capillary veins is the main reason for changes in distribution of the leukocytes. The position of the leukocytes in the blood stream of the small veins is dependent upon two chief factors; first, alterations in the circulatory rate, and second variations in the suspension stability of the blood plasma which is probably related in part, at least, to its fibrinogen content. According to this theory, a decrease in the white blood cell count results when there is a reduction in the rate of blood flow and consequently the corpuscles remain in the marginal position and many become attached loosely to the vessel wall. With an increased circulatory velocity the number of white blood cells adherent to the wall of the small veins is diminished, and as they enter again the general circulation, the leukocyte count in the peripheral blood is increased. The exact relationship of the changes in the suspension stability to the physiologic leukocytoses is less clear. Doubtless in certain pathologic conditions, such as infections, there is an increase in fibrinogen which causes a general marginal position in the veins. This, Vejlens considers, is due to an increase in the adhesiveness between the neutrophils and the vein wall, and in a reversal of the size of the red and white corpuscles. The latter is explained by the possibility that an increase in the fibrinogen content of the circulating blood causes an aggregation of red blood cells which are larger than single neutrophils or their aggregates.

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